AN INTERNATIONAL SYMPOSIUM

ON

ALDOSTERONE

Edited by

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and

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FOREWORD

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FOUR years after the famous publication of Simpson Tait Wettstein Neher von Euw and Reichstein announcing the isolation of aldosterone in crystalline form the time has come to take stock of our knowledge of this hormone

With this purpose in view the Clinique Thérapeutique of the University of Geneva organized this International Symposium on Aldosterone in June 1957 We are very grateful to Dr A Wettstein and Dr F Gross of CIBA in Basle for making such a meeting possible Our thanks go also to Dr G E W Wolstenholme of the Ciba Foundation and to J & A Churchill Ltd London for helping us to publish the papers and discussions of this Symposium

We realize more and more that among the hormones of the adrenal cortex aldosterone plays a special rôle It is the only hormone of the adrenal gland which seems to enjoy a certain freedom from pituitary control and which is influenced directly by changes in the water and electrolyte balance It is its vital rôle in the maintenance of homeostasis that makes its study so fascinating

However numerous questions still worry us We are bothered for instance by the fact that all our clinical studies are based on urinary aldosterone values that is to say on values which represent 2–3 per cent of the aldosterone secreted

We are also surprised at the conflicting clinical pictures produced by hyperaldosteronism How can we explain the fact that this hormone which in a normal person or an Addisonian causes salt and water retention should also produce in the Conn syndrome polyuria with loss of potassium and intracellular retention of sodium without water? Could it be that the Conn syndrome is something other than pure hyperaldosteronism?

The paradox of oedema with secondary hyperaldosteronism also remains to be explained Why do patients who are already swollen with water and who retain too much sodium continue to increase their aldosterone secretion?

These are some of the problems that we met in Geneva to discuss Those of us who were present may feel that a great deal remains to be done before the answers to all of them will be known but the Symposium has provided us with a point of departure for our future research programmes and it is to be hoped that, as recorded in this book it will prove interesting and stimulating to workers in this field throughout the world

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ALDOSTERONE IN URINE

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IN STUDYING the function of aldosterone in the regulation of water and salt metabolism the availability of an accurate method for the determination of this steroid in urine is of the utmost importance Many facets of the metabolic action of aldosterone under physiological and pathological conditions have been studied successfully by means of the bioassay procedures described by Simpson and Tait (1952) Johnson (1954) Singer and Venning (1953) and Bartter (1956)

However all these bioassay procedures are very time-consuming and in order to get accurate results large series of test animals have to be used Besides the available physicochemical methods there seemed to be room for a more quantitative chemical technique but no chemical method for the determination of aldosterone in urine which is specific sensitive quantitative and rapid has been described until now

The method developed in our laboratory and which is discussed here does not claim to possess all these characteristics of an ideal determination method

After it had been found that the aldosterone molecule contains three carbonyl groups we decided to try whether aldosterone could be estimated colorimetrically by reaction with 2 4-dinitrophenyl hydrazine Gornall and Macdonald (1953) had found that the absorption spectra of the 2 4 dinitrophenylhydrazones of various ketosteroids in alkaline medium differed from one another When we determined the absorption spectrum of the aldosterone derivative we found that this was definitely different from the absorption spectrum of a number of other ketosteroids e g cortisone and cortisol The more or less characteristic absorption curve which was obtained from the 2 4-dinitrophenylhydrazone of aldosterone enabled us to evaluate the homogeneity of the aldosterone fractions from urine which were purified by means of paper chromatography Furthermore a quantitative estimation of the aldosterone content of these fractions was possible

The aspects of our method discussed here are as follows

(1) purification of the extract

(2) conditions for the isolation of the coloured compound,

(3) conditions under which the colour is developed and which may contribute to the specificity of the colour reaction

PURIFICATION OF THE EXTRACT

The aldosterone containing fraction is continuously extracted with chloroform at pH 1 The chloroform extract is purified by re extraction with NaOH and water and evaporated to dryness The residue is partitioned between 70 per cent methanol and a 50/50 mixture of toluene and ligroin The methanolic solution is further purified by paper chromatography

It was found that with the paper chromatography system which we use aldosterone can be separated in one run from compound F compound E tetrahydro F and E all the less polar steroids and from a good deal of the interfering urinary pigments This system contains a mixture of 98 per cent toluene and 2 per cent octanol equilibrated with an equal amount of 50 per cent methanol and it has the great advantage that the effect of impurities on the R_F values is negligible

Fig 1 shows a chromatogram of 20 µg of compounds E and F and aldosterone giving a good separation of these three steroids The localization of aldosterone between cortisol and cortisone facilitates its identification

However it was found that the absorption curves of the 2 4-dinitrophenylhydrazine reaction product of the aldosterone fraction did not show the specific characteristics of the spectrum of the pure aldosterone derivative Furthermore when the chromatogram was sprayed with 2 4-dinitrophenylhydrazine it was found that at the place where aldosterone was localized a compound was present which gave a purplish blue colour in ammonia vapour Aldosterone could be separated from this interfering substance with the aid of a second chromatography system for which we used the B1 system (Bush 1952) in which the R_F values of cortisol aldosterone cortisone and the above mentioned impurity are 0 03 0 05 0 08 and 0 13 respectively Here again aldosterone is localized between cortisol and cortisone

Fig 2 shows u v photostats of chromatograms of cortisone cortisol aldosterone and a urinary extract from the Bush B1 system

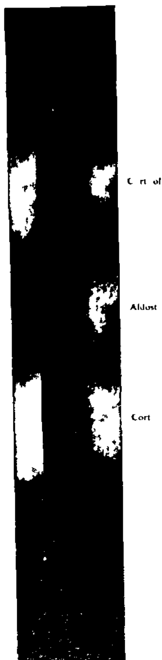
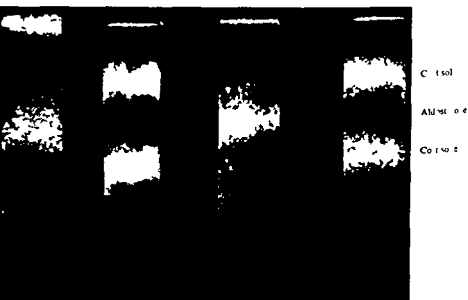


FIG 1 U V induced fluorescence on NaOH sprayed chromatogram from the toluene/octanol methanol water system



A B C D

FIG 2 U V photostat of chromatograms from the Bush B1 system

A = u ry e tract
B co t sol + cort one
C = ldosterone
D co sol + o tiso e

Table I summarizes some properties of the contaminating substance which behaved in a great number of paper chromatography systems as a single compound This substance the identity of which is not yet known was present in increased amounts in the urine of patients treated with ACTH and in one patient suffering from adrenogenital syndrome

The aldosterone fraction from the second paper chromatogram is extracted with purified ethanol and the aldosterone in the eluate determined spectrophotometrically as 2 4-dinitrophenylhydrazone

Table I

PROPERTIES OF INTERFERING COMPOUND FROM FIRST CHROMATOGRAPHY

Colour	yellow
U V absorption maximum	243 mμ
Fluorescence with NaOH	blue
Tetrazolium-blue reaction	negative
Porter–Silber reaction	negative
Zimmermann reaction	negative
2 4-Dinitrophenylhydrazone	yellow with absorption maximum in NaOH at 420 mμ with ammonia—blue

CONDITIONS FOR THE ISOLATION OF THE COLOURED COMPOUND

When 2 4-dinitrophenylhydrazine is used as a reagent in a colorimetric estimation it is necessary to remove the excess of strongly coloured reagent from the reaction mixture This can be done by the methods of Reich Crane and Sanfilippo (1953) but since these methods require about 10 mg of the phenylhydrazone they could not be used in our case In Gornall and Macdonald s (1953) study of the determination of 17 ketosteroids with 2 4-dinitrophenylhydrazine it was suggested that the excess of reagent could be destroyed by carrying out the spectrophotometrical analysis in an alkaline medium In our experience however the remaining background absorption is still very high when this method is used and therefore we tried to remove the excess of reagent in a different way It is well known that the 2 4-dinitrophenylhydrazones of various steroids (e g progesterone) are extremely insoluble in water In the procedure we are using now a precipitate of the 2 4-dinitrophenylhydrazone of aldosterone is formed by heating

the steroid with 0 1 ml of the acid reagent solution in a boiling water bath for 25 minutes The insoluble reaction product is co precipitated with benzoic acid, by the addition of sodium benzoate to the acid solution The precipitate is isolated by centrifugation and is washed twice with 2 ml of an acid washing fluid followed by centrifugation The washed precipitate is dissolved in 1 ml alcoholic sodium hydroxide and the extinction of this solution is determined at 10-mμ intervals between 380 and 600 mμ

CHARACTERISTICS OF THE ABSORPTION CURVE

The absorption spectra of the 2 4 dinitrophenylhydrazones of *aldosterone* and some other ketosteroids are shown in Fig 3

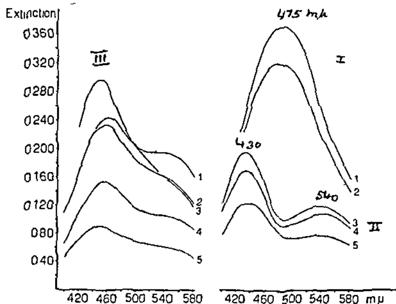


Fig 3 Extinction curves of the reaction product of 2 4-dinitrophenylhydrazine with various ketosteroids

1 = 10 μg /3 ml p ogeste e
2 = 10 μg /3 ml c rt on cetat
3 = 10 μg /3 ml cort o t rone
4 = 10 μg /3 ml testost one p pion t
5 = 3 μg /3 ml aldost rone

1 = 10 μg /3 ml cortisone
2 = 10 μg /3 ml 17-OH-cort o e
3 = 20 μg /3 ml *cis* androst one
4 = 10 μg /3 ml androst ne 17-ol 3-one
5 = 0 μg /3 ml pregn lone acet te

When standardized conditions for the formation of the coloured product are used these curves can be split clearly *in three groups* according to their shape and the place where the absorption maxi mum occurs Those in the first group have a symmetrical shape and

a maximum at approximately 475 mμ and represent the 17 hydroxy corticosteroids e g cortisol cortisone and compound S In the second group to which belong the 17 ketosteroids e g dehydro-*iso*androsterone *cis*androsterone the 3 ketosteroid androstane 17 ol 3 one and the 20-ketosteroid Δ 5 pregnen 3 ol 20 one biphasic curves with absorption maxima at 430 and 540 mμ are found In the third group we find an intermediary type of curve These curves

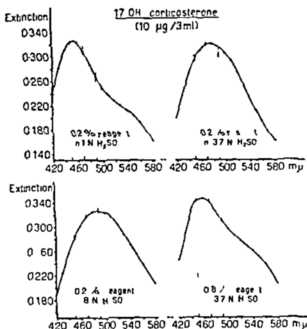


FIG 4 Extinction curves of the reaction product of 17 OH corticosterone with 2 4-dinitrophenylhydrazine prepared at different concentrations of reagent and of sulphuric acid

are given for instance by aldosterone testosterone progesterone corticosterone and cortexone The first three steroids give curves with a definite shoulder The type of curve which is obtained is partly dependent on the reaction conditions as is shown in Fig. 4

2 4-Dinitrophenylhydrazones of cortisol were prepared using different concentrations of acid and reagent At increasing concentrations of acid the absorption maximum is shifted to the longer wave lengths Under these conditions the shape of the curve also changes When an increased concentration of the 2 4-dinitrophenyl hydrazine reagent is used these changes are counteracted and the

curve which is obtained is very similar to that which was found at lower concentrations of acid A similar effect was found when cortisone and compound S were tested Steroids which do not possess a 17 hydroxy group show these changes to a much smaller extent The results of an expriment in which these same variables were tested using corticosterone are shown in Fig 5 Although small differences in the shape of the curve can be observed hardly

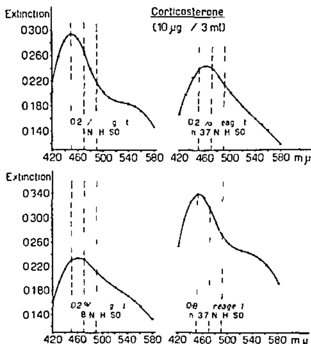


FIG 5 Extinction curves of the reaction product of corticosterone with 2 4-dinitrophenylhydrazine prepared at different concentrations of reagent and of sulphuric acid

any change in the localization of the absorption maximum was found No changes whatsoever were found with progesterone testosterone and androstane 17 ol 3 one It seems that these shifts in the absorption spectrum are only found in those steroids that possess a ketol group The effect is increased considerably when a hydroxy group is present at $C_{(17)}$ In our opinion a possible explanation is the formation of a 2 4-dinitrophenylosazone at $C_{(20)}$ and $C_{(21)}$, at high concentrations of acid *This reaction is compared to the* Porter–Silber reaction where an osazone is formed from 17 hydroxy

corticosteroids at high concentrations of acid The effect of increasing the concentration of 2 4-dinitrophenylhydrazine, which on the basis of the law of mass action seems to be in contradiction to this hypothesis can be explained by assuming that the increased concentration of the reducing agent prevents the intramolecular oxidation reduction reactions which according to Weygand precede the formation of the osazone Estimation of the nitrogen content of the various 2 4-dinitrophenylhydrazones will probably clarify the exact structures of the products which are formed

CALCULATION OF RESULTS

From the absorption spectra of the products of the reaction between 2 4-dinitrophenylhydrazine and the aldosterone fractions of a number of urines and also pure aldosterone (Fig 6) it can be concluded that

(*a*) These urinary extracts and pure aldosterone have absorption maxima at the same wave length

(*b*) The shape of the curves between 380 and 600 mμ is not in accordance with the peak of the absorption maximum

Non specific chromogens apparently are still present in the urinary extracts and these may give erroneously high values The question might be asked could a third paper chromatography achieve a further purification such that the remaining background absorption would be negligible? In our opinion such a procedure seems undesirable for the following reasons

(*a*) It is highly improbable that such a purification can be achieved by only one more chromatography

(*b*) The introduction of a third paper chromatography procedure implies a significant prolongation of the time of determination and may increase the loss of aldosterone considerably

(*c*) As indicated below it seems possible to correct for the non specific chromogens which are still present

Therefore we did not carry out further purification procedures and we tried to calculate the aldosterone content of the urinary fractions from the absorption curves which were obtained We are aware that this introduces some risk but in our opinion the method as indicated gives sufficiently reliable results to justify its use in the study of the problems discussed in the introduction to this paper

The correction for background absorption can only be made if the shape of the absorption curve from these non specific chromogens

is known For obvious reasons it is impossible to establish in any individual case the shape of the correction curve and therefore arbitrary correction procedures have to be followed

In Fig 6 the extinction values at 500 mμ are plotted at the base The extinction curves for the various urinary fractions between 420 and 500 mμ in this case approach to the base The curve which runs almost parallel with the base is from the aldosterone fraction of

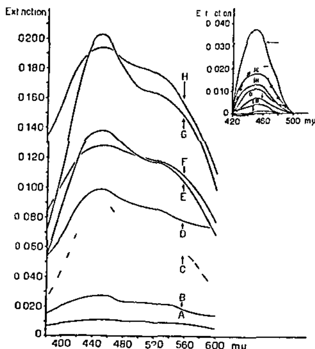


Fig 6 Extinction curves of various urinary extracts and pure aldosterone (C)

urine from an Addisonian patient Therefore it is highly probable that in this case the curve shown is the extinction curve of the non specific chromogens This observation seems to justify correction for these non specific chromogens by subtraction of the extinction at 420 or 500 mμ from the extinction at the absorption maximum Comparison of these differences between the standard and unknown sample permits the calculation of the aldosterone content of the unknown sample In making this correction we prefer to use the extinction at 500 mμ and not the 420 mμ reading, because the

absorption curve of the 2 4 dinitrophenylhydrazones of cortisone and of cortisol have absorption maxima at 480 mμ and give the same extinction values at 460 and 500 mμ This implies that in cases where the urinary aldosterone fraction is contaminated with small amounts of these compounds the same correction formula is valid

RESULTS

Our data on the reproducibility of the method described above are summarized in Table II When we consider the great number of

Table II

RESULTS OF DUPLICATE DETERMINATIONS OF ALDOSTERONE IN URINE

Patient	*Date*	*μg /24 hrs of aldosterone*		*Mean*
		I	II	
Ba	Jan 1957	2 0	3 5	2 8
Fra	Oct 1956	2 0	2 5	2 3
Bay	Oct 1956	2 5	3 5	3 0
Es	May 1956	4 5	7 0	5 8
Ee	Jan 1957	7 5	7 5	7 5
Ee	Jan 1957	12 5	17 5	15 0
St	Jan 1957	19 5	20 0	19 8
Ee	Jan 1957	23 5	35 0	29 3
Bo	Aug 1956	46 0	51 0	48 5

steps involved in this method the differences which were found between duplicate determinations seem to be acceptable Table III

Table III

THE EXCRETION OF ALDOSTERONE IN NORMAL INDIVIDUALS IN μg /24 HRS

Males		*Females*	
Mol	5 5	Ba2	2 5
Ka	6 0	Bu	2 5
Es	6 0	La	3 0
Ch	6 0	Ol	3 5
Co	6 5	Ba1	3 5
Br	6 5	Lee	4 0
Mo2	9 5	Ke	4 5
Gr	13 0	Bu	4 5
Am	13 0	La	5 5
		Po	6 0
		Al	6 5
		Vo	8 0
Mean	8 (5 5–13 0)		4 5 (2 5–8 0)

shows the values which were obtained in normal individuals These values are within the ranges which have been found by a number of other investigators although they are somewhat higher than those found by Luetscher and Axelrad (1954) The number of our observations in normal individuals is too small to decide whether there exist significant differences in the aldosterone output of males and females We have been unable to carry out extensive recovery experiments in order to test our method further due to the fact that we had only 200 μg of pure aldosterone available for this study

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[Discussion of this paper was postponed until after the paper by Dr Neher —EDS]

ALDOSTERONE AND OTHER ADRENOCORTICAL HORMONES IN HUMAN ADRENALS AND ADRENAL TUMOURS

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INTRODUCTION

Already much effort has been made to characterize the functional state of the adrenals by determination of the corticosteroid hormones in the blood and urine and to correlate it in this way with the clinical symptoms Thanks to new methods of determination of individual corticosteroids one has in diseased conditions already been able to form a concrete picture about hypo- or hyperfunction of the adrenals and to set up certain demarcations These differentiations however are somewhat crude in view of the six corticosteroid hormones observed in man—cortisol cortisone aldosterone corticosterone 11 dehydrocorticosterone (Kendall s A) and Reichstein s substance S (17 hydroxycortexone)—when one considers that their qualitative and quantitative variation must lead to an extremely diversified possibility of secretion One very seldom has to deal with a single hormone in cases of hypo- and hyper secretion but usually with mixed forms Therefore the present survey covers the whole of the known corticosteroid hormones

So far the finer differences in adrenal function the clinical significance of which is still obscure are very difficult to detect by means of urine or blood determinations (cf Neher 1957) For instance only extremely small amounts of the hormones excreted in the urine are in unmetabolized and unconjugated forms e g less than 1 per cent of cortisone (Peterson *et al* 1955) then again the various hormones are catabolized in the blood at varying rates (Ayres *et al* 1957 Migeon *et al* 1956*a* Touchstone *et al* 1957) and are eliminated by different routes (Hellman *et al* 1956 Migeon *et al* 1956 *a* and *b*)

It therefore seemed of interest to us to determine the cont nt of corticosteroid hormones during various endocrine disturbances at the production source itself in separate adrenals and adrenal

tumours We were of course aware that such determinations like those in the blood only represent a quick picture that in addition can be out of focus because of the associated condition of removal of the gland They do however permit the estimation of hormone content e g in the left and right adrenal, or in adrenal and adhering tumour, and the determination of the extent to which hormone production in various tissues is co ordinated i e compensated or proceeding independently We shall present here our first results obtained with 20 adrenals or adrenal tumours (cf Neher 1956) The material comes from operations or autopsy

METHODS

Material from operations was sent to us deep frozen and was maintained in this condition for anything from a few days to several weeks Material from autopsy was obtained as rapidly as possible not longer than 1–2 hours after death Our procedure was as follows

(a) The chopped tissue was homogenized or minced in saline

(b) The suspension was poured into 10–20 times the volume of 80 per cent acetone and allowed to stand several hours with occasional stirring

(c) The residue was filtered off and washed with 80 per cent acetone and the acetone solution was concentrated *in vacuo*

(d) The aqueous suspension was extracted with chloroform and the chloroform solution was concentrated

(e) The chloroform extract was dissolved to 1 per cent in petroleum ether and extracted four times with half the volume of 60 per cent methanol (addition of NaCl)

(f) The aqueous methanol solution was concentrated *in vacuo* to half its volume and extracted with chloroform

(g) The evaporated chloroform extract was chromatographed on 20 times the amount of silica gel with a chloroform acetone mixture The corticosteroid fraction was eluted with chloroform acetone in the ratio 1 1

(h) This fraction (about 1–3 mg /adrenal) was paper chromato graphed as illustrated in Fig 1

(i) Identification and semiquantitative determination of the corticosteroids was carried out according to the R_F values in various systems tetrazolium blue u v absorption soda fluorescence phosphoric acid (cf in this connexion aldosterone determination Neher and Wettstein 1955, 1956*a*) error about ± 20 per cent

At first following removal of the acetone in step (*c*) the aqueous solution was defatted directly with petroleum ether This can however, lead to a loss of corticosterone since it can go partially into the petroleum ether in presence of much lipoid material In the extracts of separate adrenals or adrenal tumours it could be demonstrated that besides the hormones at least eight other steroidal compounds were present but these will not be surveyed here

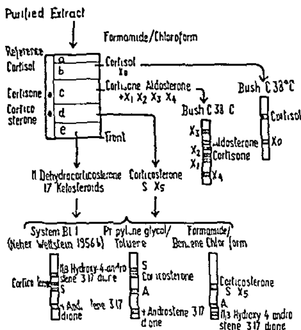


FIG 1 Scheme of paper chromatographic fractionation of adrenal extracts

RESULTS AND DISCUSSION

It is of course rather difficult to obtain so-called normal adrenals which give comparison values The only people who could be considered as a source of these normal adrenals were those without endocrine disturbances whose adrenals could be removed not later than 1–2 hours after sudden death From operation no adrenals were available that could with certainty be considered normal For this reason it has not been possible up to the present to obtain a larger number of comparison values than are listed in

Table I It is possible that only case 1/1 can be considered as absolutely normal Since the adrenal function is defined both by the qualitative and quantitative composition of hormones produced

Table I

ADRENOCORTICAL HORMONES IN SUBJECTS WITH NORMAL ADRENAL FUNCTION

Cas	*1/1 ○ 0 ɜ t affic ac d t (sul id)*		*1/2 ♀ 77 y s † a t ardiac fallu e*		*1/3 ♂ 21 yrs s l ld f d hang d 12 h aft r †*		*Val s f om th l t t*	
Cort t ds	*left* 5 15 g	*ight* 4 63 g	*l ft* 4 91 g	*ight* 5 14 g	*l ft* 3 71 g	*right* 3 4 g	*6 ad e l (d lts) (Huds a d Lomb d 1955)*	*Foetal adrenal glands (Bloch t al 1956)*
C tisol								
µg total	0	11 5	8 0	11 2	<0 5	<0 5		
µg /g. tissue	3 9	5	1 53	2 18	<0 13	<0 15	2 3-5 5	0 8
	55 9	69 5	13 3	37 1	—	—		
C tiso								
µg total	0 5	0 2	0 25	2 0	0	0 75		
µg. g tissu	0 1	0 04	0 05	0 39	0	0 17	—	
/	1 4	1 2	0 4	6 6	—	—		
Ald t e								
µg total	0 25	0 25	<0 3	2 0	0	<0 25		
µg /g. t s ue	0 05	0 05	<0 06	0 39	0	<0 07	—	
	0 7	1 5	—	6 6	—	—		
Co ticost n								
µg total	15 0	4 6	52	15	0 8	3 0		
µg. g tissue	2 9	1 0	10 6	2 92	0 2	0 88	—	0 8
/	42 0	27 5	86 3	49 7	—	—		
Other H mo								
µg. tot l								
µg /g ti			S (+)	0				
Ratio I C tl ol / C t o one	1 3	2 5	0 15	0 75	—	—		1 0
Rat o II Cor l ol / Ald t on	80	46	>77	5 6	—	—		

the values are expressed in µg per gland or tumour in µg per g tissue as well as by the percentage of the single hormones for the total hormone quantity and the ratio cortisol/corticosterone (I) or cortisol/aldosterone (II)

Case 1/1 can be considered as normal Cortisol as expected is present in the largest amount (55 9–69 5 per cent) cortisone and

aldosterone only to a slight extent (1 2–1 4 and 0 7–1 5 per cent) while corticosterone occurred again in rather large amounts (42 0–27 5 per cent) in any case in larger amounts than one might have expected from the normal values in peripheral blood or in urine Ratio I is 1 3 and 2 5 and ratio II is 80 and 46 for the left and right glands respectively On the whole one gets the impression that both adrenals have functioned very similarly and that certain compensatory phenomena within this pair of adrenals must be assumed for cortisol as well as for corticosterone

The age and mode of death of case 1/2 are indeed very different from the previous one (significant sex differences in blood and urine corticosteroids have not so far been discovered) it was nevertheless a normal adrenal function All the more surprising then is the rise in corticosterone over cortisol and in both adrenals too a fact that is shown very clearly by ratio I Compared with case 1/1 there seems to be a marked compensation of aldosterone between the left and right adrenal It remains for further experiments to decide whether this high corticosterone content is a normal occurrence or only a chance observation or whether it has something to do with the disease state of the patient

Case 1/3 is only shown as a consequence of late acquisition of material it shows that a relatively prolonged postmortem period is associated with practically complete disappearance of the corticosteroid hormones

Several cases of patients with primary hyperaldosteronism from whom adrenal tumours were removed and studied are listed in Table II In accordance with expectations the aldosterone content as compared with normal adrenals was found to be significantly (from 3 to 40 times) higher Still higher values have been reported in the literature (Conn and Louis 1955 Eales and Linder 1956) If in addition one adds in the probable production of the remaining adrenal tissue one arrives at a very considerable overproduction of aldosterone In cases 2/2 and 2/3*a* the concentration of the other hormones corresponds to that of the normal adrenal tissue while in case 2/1 (cf Mader and Iseri 1955) in addition to aldosterone cortisol is also a little raised while corticosterone appears to be tremendously increased This is probably another case of a mixed syndrome the clinical designation of which as primary aldosteronism does not conform completely to the analytical findings The determination of content in the adrenals normally remaining in the patient would

certainly be very interesting here Such a study was possible in case 2/3 (*a* and *b*) in which a significant difference both in aldosterone and cortisol content between the tumour and the corresponding adrenal could be established While adenoma presents the typical picture of

Table II

ADRENOCORTICAL HORMONES IN TUMOURS OF PATIENTS WITH PRIMARY ALDOSTERONISM

Cas *Corticoste oids*		2 1 *T m u from l ft ad nal* 6 5 g (*USA*)	2/2 *Ad ma from ight ad nal* 6 g (*Japan*)	2/3a *Adenoma f om l ft adre al* 4 9 g (*Swed*)	2/3b *l ft d nal (witho t ad n ma)* 5 5 g	*Val es fr m the l t t e* (*C nn and Lo ls* 1955) *Ad oma* (r ? g)	*Val es fr m the l t t e* (*Eales and Ll de* 1956) *Ad oma* (l 27 g)
C tis l	µg total µg /g tissue	58 5 9 0 21 0	21 3 5 76 9	19 2 4 0 53 2	67 7 12 3 88 1		5 4 0 2 —
C tl on	µg t tal µg /g tiss e /	0 0 —	<6 0 <1 0 —	<1 5 0 —	2 47 0 45 3 2		
Aldost one	µg total µg /g tis /	9 1 1 4 3 3	6 3 1 05 23 1	5 29 1 08 14 6	0 77 0 14 1 0	8 7	151 5 6 —
C t t	µg total µg /g t /	212 32 5 75 7	<8 4 <1 4 —	9 8 2 0 27 1	5 90 1 07 7 7		
Other Horm ne (11 Dehyd o-cort co t one)	µg tot l µg /g tissue /			1 86 0 38 5 1			
Ratio I $\frac{C\ tis\ l}{C\ tic\ t\ o}$		0 28	>2 5	2 0	11 5	—	—
Ratio II $\frac{Cortisol}{Aldost\ one}$		6 4	3 3	3 6	88	—	0 04

primary aldosteronism the remaining gland fits rather to a Cushing type Seen as a whole therefore it is again a case of a mixed form In addition, there seems to be no compensation here in the secretion of individual hormones by tumour and adrenal especially as regards the aldosterone which despite the high content in the tumour shows at least a normal concentration in the adrenal

Results from patients with adrenal hyperfunction caused by tumour or hyperplasia are listed again in Table III Case 3/1 can be considered as a typical Cushing s syndrome when the total

Table III

ADRENOCORTICAL HORMONES IN PATIENTS WITH ADRENAL HYPERFUNCTION (ADRENAL TUMOUR OR HYPERPLASIA)

Cas *Corticost oid*	3 1 ♀ 30 *yr* *Cushi g Sy dr m adr nal tum ur l ft* *about* 100 *g*	3 2 *A A* *Cushing s Syndrom adr nal t m ur* 9 86 *g*	3-3 ♀ 1 1/2 *yrs* *l llizing adr nog t l t nd om adr nal carcinoma* 142 *g*	3 4 ♂ F *bil t al adr nal hyperplasia l ft and ight adrenal* 11 38 *g*	3 5 ♀ 25 *yrs* *C shing's di as hyperplasia f right adr nal* 11 5 *g*
C rtisol μg total μg /g tiss e	160 1 6 91 5	95 9 6 23 8	41 6 0 29 74 0	60 0 5 3 56 2	23 4 2 03 81 2
C rtiso μg total μg /g tissue	7 7 0 08 4 4	290 29 4 72 7	9 6 0 07 17 1	4 0 0 35 3 7	0 69 0 06 2 4
Aldo terone μg. total μg /g tissue	3 9 0 04 2 2	2 8 0 8 0 7	1 0 tr ce 1 8	2 8 0 25 2 6	1 04 0 09 3 6
Corti o t μg total μg /g. tis ue	2 9 0 03 1 6	<8 <0 8 —	4 0 0 01 7 1	40 0 3 5 37 5	3 68 0 32 12 8
Othe Horm es μg. total μg /g. tissue	"S (+) A +(0 3 ?)	S 11 1 12 2 8			
Rat I *C rt l* / *Corti t one*	55	>12	10 5	1 5	6 4
Rat o II *Co ti l* / *Aldo t one*	41	34	42	21	22

amount of aldosterone is also slightly raised but corticosterone on the other hand is lowered so that ratios I and II are about the same Expressed in μg /g tissue a low value is obtained in this case for all hormones The tumour of case 3/2 also a Cushing s syndrome exhibits once again quite a different picture Here

certaınly be very ınterestıng here Such a study was possıble ın case 2/3 (*a* and *b*) ın whıch a sıgnıficant dıfference both ın aldosterone and cortısol content between the tumour and the correspondıng adrenal could be establıshed Whıle adenoma presents the typıcal pıcture of

Table II

ADRENOCORTICAL HORMONES IN TUMOURS OF PATIENTS WITH PRIMARY ALDOSTERONISM

Case *Cortıcost roıds*		2 1 *Tumour f om l ft ad enal* 6 5 g (*USA*)	2/2 *Ad noma f m ıght ad e l* 6 g (*Jap*)	2/3a *Ade ma f om left adre l* 4 9 g (*Sw de*)	/3b *l ft ad e l (ıth t ad noma)* 5 5 g	*Val e f m the lı at* (*Co n and Lo ıs* 1955) *Ad m* (r 7 g)	(*Eal s and Lı der* 1956) *Ade ma* (l 27 g)
Co tısol	µg total µg /g tıss e /	58 5 9 0 21 0	1 3 5 76 9	19 2 4 0 53 2	67 7 12 3 88 1		5 4 0 2 —
C tı	µg total µg /g tıss	0 0 —	<6 0 <1 0 —	<1 5 0 —	2 47 0 45 3 2		
Aldost one	µg total µg /g tıssue /	9 1 1 4 3 3	6 3 1 05 23 1	5 29 1 08 14 6	0 77 0 14 1 0	8 7	151 5 6 —
Co t costı o	µg total µg /g t s /	212 32 5 75 7	<8 4 <1 4 —	9 8 2 0 27 1	5 90 1 07 7 7		
Oth H m nes (11 Dehydro- c rt costerone)	µg total µg /g tıs /			1 86 0 38 5 1			
Rat o I $\frac{Co\ tıs\ l}{C\ t\ \ t\ o\ e}$		0 8	>2 5	2 0	11 5	—	—
Ratıo II $\frac{C\ tı\ l}{Ald\ t\ one}$		6 4	3 3	3 6	88	—	0 04

prımary aldosteronısm the remaınıng gland fits rather to a Cushıng type Seen as a whole therefore ıt ıs agaın a case of a mıxed form In addıtıon there seems to be no compensatıon here ın the secretıon of ındıvıdual hormones by tumour and adrenal especıally as regards the aldosterone whıch despıte the hıgh content ın the tumour shows at least a normal concentratıon ın the adrenal

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Table III

ADRENOCORTICAL HORMONES IN PATIENTS WITH ADRENAL HYPERFUNCTION (ADRENAL TUMOUR OR HYPERPLASIA)

C e *Cortic t ids*	3 1 ♀ 30 y s "Cushi g s Syndrom adr nal tumour l ft *about* 100 g	3 2 A.A Cu hi g s S ndrom ad enal t mour 9 86 g	3/3 ♀ 1 1/2 yrs l lli ing adr og ital synd om adr nal car inom 142 g	3/4 ♂ F bilat l adrenal h perplasia l ft and right adr nal 11 38 g	3 5 ♀ 25 yrs Cushi g's dis as h perplasia of ight adrenal 11 5 g
Cortis l μg total μg/g tissu /	160 1 6 91 5	95 9 6 23 8	41 6 0 9 74 0	60 0 5 3 56 2	23 4 2 03 81 2
Cortiso μg total μg/g tiss e /	7 7 0 08 4 4	790 29 4 72 7	9 6 0 07 17 1	4 0 0 35 3 7	0 69 0 06 2 4
Aldo te e μg total μg/g tis e	3 9 0 04 2 2	2 8 0 28 0 7	1 0 trace 1 8	2 8 0 25 2 6	1 04 0 09 3 6
Cortl o t μg. total μg/g tissue	2 9 0 03 1 6	<8 <0 8 —	4 0 0 03 7 1	40 0 3 5 37 5	3 68 0 32 12 8
Othe H rmo μg. total μg/g tissue /	"S (+) A +(0 3/g)	'S 11 1 12 2 8			
R tio I *Cortis l* / *Cort teron*	55	>1	10 5	1 5	6 4
Rati II *Co ti l* / *Aldo t*	41	34	42	21	22

amount of aldosterone is also slightly raised but corticosterone on the oth r hand is lowered so that ratios I and II are about the same Expressed in μg/g tissue a low value is obtained in this case for all hormones The tumour of case 3/2 also a Cushing s syndrome exhibits once again quite a different picture Here

firstly the interconversion between cortisol and cortisone seems to be disturbed so that an enormous cortisone value is obtained and secondly the presence of a relatively large amount of Reichstein s substance S is noticeable this usually occurring in quite small amounts or even not being observable at all

A further case 3/3 concerns a virilizing adrenogenital syndrome that had been treated with cortisone to no avail Here the ratio cortisol/corticosterone exclusively is somewhat raised (10 5) probably at the expense of corticosterone (7 1 per cent) Otherwise the overproduction of the adrenal hormones by the carcinoma is due only to its high weight [See Anliker Rohr and Marti (1956) for a case of an 800-g virilizing adrenal tumour with a relatively low corticosteroid production]

The production capacity of the hyperplastic adrenal of case 3/4 relates to all hormones in about the same proportion where both adrenals were worked up together Here hyperfunction results in a somewhat raised value in µg /g tissue as well as in a raised adrenal weight This cannot be said for the next case 3/5 where the adrenals were once again studied separately the concentration in µg /g appears to be quite normal here and the hyperfunction must be attributed exclusively to the raised weight of the producing tissue

Finally several other cases of disturbed adrenal function due to metastases or necrosis are listed in Table IV Case 4/1 referred to a patient who showed no distinct endocrine disturbances clinically Besides partial hypotension neurological disturbances were the most obvious Autopsy showed a distinctly enlarged left adrenal (32 g) due to tumorous infiltration of the medullar and cortical sinusoids The cortical cells seemed to have atrophied probably because of compression The same tumour cells i e metastases were found in pituitary and brain From evaluation of the analytical results of the left adrenal alone (the right adrenal was unfortunately not available to us) one might have immediately diagnosed primary aldosteronism (ratio II low with normal cortisol content) That no such manifestations were obvious is difficult to explain in view of the generalized tumorous infiltration but it seems possible that the raised aldosterone production had been compensated by other partially antagonizing hormones

The analytical results of case 4/2 with the exception of the noticeably higher content of substance S would imply quite a normal adrenal function The earlier history of this case is however

rather interesting The patient had strong electrolyte disturbances alkalosis hypokalemia and hypernatraemia and besides the obvious overproduction of the adrenals probably lung cancer with metastases

Table II

ADRENOCORTICAL STEROIDS IN PATIENTS WITH ABNORMAL ADRENAL FUNCTION (METASTASES NECROSIS)

Cas *Corticost roids*	4 1 ♂ 49 yrs *Slight adr nal hyperfunction tumorous infilt tion of left adrenal* 3 g	4 ♀ 60 yrs *Hypercorti ism" carci noma of the lung left adrenal metastases* *l* 13 g	*r* 10 g	4 3 ♀ 5 yrs *"Cushing Syndrome oper ti e e plor tion of left adr nal* 5 5 g	3 5 right adrenal 11 5 g *(f om Tabl III)*
Cortisol µg. total µg./g tissue	4 5 0 77 61 5	23 5 1 81 65 5	39 1 3 91 78 9	1 38 0 23 3 9	3 4 2 03 81 2
Cortisone µg. total µg./g. tissue	8 1 0 25 0 4	1 3 0 1 3 6	1 8 0 18 3 6	0 0 —	0 69 0 06 2 4
Aldosteron µg. total µg./g. tissue	5 2 0 16 13 1	0 52 0 04 1 5	0 7 0 07 1 4	33 3 6 07 95 0	1 04 0 09 3 6
Cortico ter e µg t tal µg./g tiss e	2 0 0 06 5 0	4 16 0 33 11 6	8 0 0 80 16 1	0 39 0 07 1 1	3 68 0 32 12 8
Other Hormones µg. total µg./g tiss e	"A" (+)	"S" 6 4 0 49 17 8	"S" (+)		
Ratio I *Cortis l / Cort i one*	12 3	5 6	4 9	3 5	6 4
R tio II *Cortis l / Ald t ron*	4 7	45	56	0 04	2

Nevertheless the electrolyte disturbances disappeared suddenly and death followed shortly afterwards Autopsy showed metastases of a lung carcinoma especially in the left adrenal and a diffuse partly adenomatous hyperplasia of the zona fasciculata While the latter can still be recognized in the somewhat raised cortisol value especially in the right adrenal the aldosterone concentration in

accordance with the spontaneous remission of the electrolyte disturbances is practically normal The earlier electrolyte disturbances might have corresponded in fact to a raised aldosterone production but this seems to have been partly destroyed by metastases i e in this case to have become normalized

A curious picture is presented by the two adrenals of case 4/3 whose right adrenal has already been mentioned (3/5) It displayed a hyperplasia of the adrenal cortex an adenoma of the anterior pituitary lobe typical of Cushing s syndrome About six weeks before death from lung embolia the left adrenal was surgically exposed because of suspected tumour but nothing abnormal was found the right adrenal was not exposed The autopsy showed besides hyperplasia of the right adrenal (11 5 g) a subtotal necrosis of the hyperplastic left adrenal (5 5 g) that may have been caused by the previous exposure Where the whole cortex had not yet been necrotic or replaced by granulation tissue the fasciculata seemed to be almost completely missing on the other hand glomerulosa like cortical epithelia predominated This finding corresponds excellently with the analytical results and supports the contention that aldosterone is also formed in man in the glomerulosa and cortisol in the fasciculata the latter could also be responsible for corticosterone It remains obscure whether the selectivity of this necrosis with maintenance of the aldosterone production is purely chance or conditioned by other circumstances such as operative trauma In any case the adrenals were not able to compensate the hormone secretion internally since finally one adrenal was typical of primary aldosteronism the other of hyperplasia

The cases examined arranged according to adrenals and tumours are compared synoptically in Table V on the basis of the single values or even better of ratios I and II In part clear differences of separate types of hyperfunction can be established though the extensive mixed forms cause a large amount of overlapping Instead of cortisol or corticosterone the use of cortisol + cortisone or corticosterone + 11 dehydrocorticosterone would probably be better suited for the ratios The given ratio of cortisol corticosterone = 0 72–1 6 for normal adrenal pairs tallies well with that found in adrenal venous blood of about 0 5–5 (cf in this connexion Bush 1955 Sweat 1956 Tamm 1956 Neher 1957) In comparison with this the ratio in peripheral blood attains to about 5–30 a fact that supports the theory of a rapid metabolism of corticosterone as com

of course still much too small to allow us to draw extensive conclusions between production capacity of the adrenal and clinical manifestation Neither is this in our opinion advisable inasmuch as not all adrenal hormones are yet known (Desaulles Neher and Wettstein 1956) Still these experiments show that besides aldosterone and cortisol equal notice should be taken of at least corticosterone and especially in cases of adrenal tumours substance S (cf Bongiovanni and Eberlein 1955) the significance of which two hormones is by no means clear

Acknowledgements

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pared with cortisol, this metabolism having been confirmed by tracer methods (Migeon *et al* 1956*a*) The comparison of the correspond ing ratio cortisol aldosterone in normal adrenals (*9 6–63*) and in peripheral blood (about 50–400) leads one to suppose that aldosterone too is more rapidly metabolized or more rapidly excreted from the

Table V

COMPARISON OF CONTENTS OF ADRENOCORTICAL HORMONES IN HUMAN ADRENALS AND ADRENAL TUMOURS

Adrenal function	*Cortisol* µg. t tal ( )	*Cortis ne* µg t tal ( )	*Aldo t one* µg t tal ( )	*Corti o- t rone* µg total ( )	*Sub-tance S* µg total ( )	*I* Ratio *Cortisol* / *C ti ost*	*II* *Corti ol* / *Aldoste on*
I Singl Adr nals No mal	8– 0 (13 3–69 5)	0 2 2 0 (0 4–6 6)	<0 3 2 0 (0 7–6 6)	4 6–52 (27 5–86 3)	0–(+) —	ingle dre als 0 15–2 5 pairs f ad n ls 0 72 1 6	singl adre ls 5 6–80 p irs of drena s 9 6–63
Primary Aldosteronism	67 7 (88 1)	2 47 (3 2)	0 77 (1 0)	5 90 (7 7)	0	11 5	88
Hyperplasia	3 4–30 (56 2–81 2)	0 69–2 0 (2 4–3 7)	1 04–1 4 (2 6–3 6)	3 68– 0 (12 8–37 5)	0	1 5–6 4	21 22
Necrosis	1 38 (3 9)	0 —	33 3 (95 0)	0 39 (1 1)	0	3 5	0 04
II Tumo rs Primary Aldoste nism	19 2 58 5 (21 0–76 9)	0	5 29–9 1 (3 3–23 1)	<8 212 (0–75 7)	0	0 8–2 0	3 3–6 4
Hyperfuncti n	41 6–160 (23 8–91 5)	7 7 290 (4 4–72 7)	1 0–3 9 (0 7 2 2)	<8–4 0 (0–7 1)	0–11 (0–2 8)	10 5–55	34–42
Meta tases	23 5–39 1 (61 5–78 9)	1 3–8 1 (3 6– 0 4)	0 5 5 2 (1 4–13 1)	2 0–8 0 (5 0–16 1)	0–6 4 (0–17 8)	4 9–12 3	4 7–56

blood than cortisol this also having been made probable recently by means of tracer studies (Ayres *et al* 1957)

In conclusion it may be said that the determination of individual corticosteroid hormones in the adrenals or tumours may reveal interesting details with reference to adrenal function under various pathological conditions this concerns besides the general situation of quantitative and qualitative production both the relative pro portions of production of hormones from adrenal pairs and that from separate adrenals and their tumours The material examined is

Moolenaar It is possible of course We thought about introducing a third chromatogram but as I have already mentioned this would prolong our determination considerably and we would run the risk of losing some aldosterone in every paper chromatographic separation Furthermore if you use a third chromatogram you do not know that this removes every thing which may interfere with your reaction

Neher We are probably lucky that European urine extracts are less complicated than American urine extracts Dr Genest in Montreal for instance reported on a compound III which was running very similarly to aldosterone and which seems to derive from citrus fruits Using the Eberlein Bongiovanni system he found that this compound III can be separated from aldosterone

S A S Tait Prof Gray and his co-workers have also noticed such a compound which was often present in the urine of pregnant women and in subjects engaged in hot room experiments It was finally found by his group that this compound which has chemical properties very similar to those of the compound described by Genest came from orange juice and was not of endocrinological origin

Giroud At a recent meeting of the Endocrine Society in New York Dr Nowaczynski of Dr Genest s group presented data showing that he has established the identity of compound III with that of Prof Gray s compound. It is a fact that this compound can be found in great amounts in citrous juices but Dr Nowaczynski has also observed that it is excreted in significant amounts in the urine of subjects fed on a compound III free diet. Furthermore two patients on this diet who were receiving high potassium intake showed an increased urinary excretion of compound III

Regarding the problem of contaminants in North American urines and the difficulty of measuring urinary aldosterone by a chemical method I feel that I should quote here the important contribution of Dr Genest The method which he has been using during the past years (1957 *Canad J Biochem Physiol* 35, 425) involves a first purification of the crude neutral extract of urine on a silica gel column followed by a stepwise separation of aldosterone in the propylene glycol toluene system of Zaffaroni the *Eberlein Bongiovanni E2B system, and finally in the Bush B5 system.* After elution from the third chromatogram aldosterone is quantitatively measured both by its absorption at 239 mμ and by the tetrazolium blue reaction Dr Genest feels that such a sequence of chromatographic systems is compulsory in order to separate aldosterone from up to seven compounds whose property to absorb u v light or to reduce tetrazolium blue would interfere with its physicochemical estimation

J F Tait I would like to defend Dr Moolenaar s method at least re garding its application to normal urine He sent us some extracts from the final chromatogram of his method We acetylated them and ran them on the Bush B3 system As shown by soda fluorescence only small amounts of impurities were present and the quantity of aldosterone diacetate as estimated by a fluorimeter corresponds rather well with that obtained by Dr Moolenaar using the Gornall reaction before acetylating the extract However as these were extracts from normal urine there is still a possi bility as Dr Neher suggests of lack of specificity for pathological urine

Wolff We compared urinary aldosterone in healthy females and healthy males on a constant sodium and water intake and we could not find *significant differences*

In this physicochemical determination some supplementary information often seems desirable from a qualitative point of view Besides the aldosterone characteristics already mentioned (BT positive soda fluorescence same running as cortisol in the second chromatogram in the Bush C system) we suggest a third chromatogram in another system a negative phosphate test (if there is less than 20 μg aldosterone available) and chromatography of the 11 18 acetylated product.

Dr Moolenaar spoke of finding a blue fluorescent substance between E and F on the Bush system Elution of this or a similar substance has been reported (Eades C H Jr Pollack R L and King J S Jr (1954) *Fed Proc* 13 201) On acid hydrolysis the authors obtained a substance which they said was a steroid substance and an amino acid (glycine) so they claimed that it was a steroid amino acid conjugate

Brooks We have found a pigment which fluoresces blue in the sodium hydroxide reaction with u v light and for that reason we have added another stage to the Neher and Wettstein method that of acetylation of the cortisol region from the Bush C chromatogram and running the acetylated material in another system intermediate between the Bush A and B1 systems we then get a very clean pigment free product Dr Moolenaar have you tried the 2 4 dinitrophenylhydrazine absorption curve of the aldosterone diacetate?

Moolenaar Yes

Brooks Is it characteristic?

Moolenaar I think so

Neher We have found many substances in urinary extracts which fluoresce blue or greenish or with some other colour particularly in pathological cases I wonder why you used for your separation procedure two systems in which aldosterone has the same running rate relative to cortisone and cortisol Is there any special reason?

Moolenaar When we did these experiments we had almost no aldosterone available so we could not use a control strip with aldosterone and could scarcely localize the aldosterone on the chromatogram We were looking for a system where aldosterone is easily localized between two carrier substances and therefore we were happy with our two systems where aldosterone was running between cortisol and cortisone

Neher Do you determine aldosterone only by the reaction with 2 4 dinitrophenylhydrazine? There may be some possibility of contamination of your aldosterone eluate by other steroids with reactive keto groups I wonder if the sole reaction with 2 4 dinitrophenylhydrazine is sufficiently specific

Moolenaar We thought about that but for several reasons we are not afraid that we are determining ketosteroids instead of aldosterone The ketosteroids that we tested for instance 17 ketosteroids run mostly at the front of the chromatogram they have high running properties in these systems Most ketosteroids are removed even in the partitioning which we do before chromatography Another point is that none of the keto steroids shown in our picture gave the characteristic curve which we found for aldosterone Furthermore the phenylhydrazone and soda fluorescence tests were both positive for the aldosterone fraction

Neher Yes but there may be especially in pathological cases other ketosteroids which are not 17 ketosteroids and which may have a higher polarity e g 20 ketosteroids There may be steroids present without reducing or u v absorbing properties

Moolenaar It is possible of course. We thought about introducing a third chromatogram but as I have already mentioned this would prolong our determination considerably and we would run the risk of losing some aldosterone in every paper chromatographic separation Furthermore if you use a third chromatogram you do not know that this removes every thing which may interfere with your reaction

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Wolff We compared urinary aldosterone in healthy females and healthy males on a constant sodium and water intake and we could not find significant differences

Moolenaar We did find differences between males and females but we have not enough data to say that there are significant differences

Baulieu Giroud and co-workers (Giroud C J P Stachenko J and Venning E H (1956) *Proc Soc exp Biol N Y* 92, 154) and Ayres and co-workers (Ayres P J Gould R P Simpson S A S and Tait J F (1956) *Biochem J* 63 19P) have shown the part played by the zona glomerulosa in the biogenesis of aldosterone in the rat and the ox Garrod and co-workers (1956 *IV Int Congr Med*) have observed the excessive production of aldosterone by two Conn tumours which histologically resembled the zona glomerulosa

We report here (Baulieu E E Tourneur R and Jayle M F (1957) Unpublished observations) two cases with androgenic tumours kalaemia being normal only one is hypertensive and secretes an excess of aldosterone

Table I

Two Cases of Virilizing Adrenal Tumour

	Case D (1) (*fem age 2 years*)	*Case A* (2) (*fem age 30 years*)
Adrenal histology (3)	benign	malignant
Hypertension	+ +	0
Plasma potassium	normal	normal
17 Ketosteroids mg /24 hours (4)	131 0	305 0
17 Hydroxysteroids mg /24 hours (5)	13 5	14 6
Cortisol µg /24 hours (6)	120 0	15 0
Cortisone µg /24 hours (6)	35 0	5 0
Aldosterone µg /24 hours (6)	18 0	2 0

(1) Service P Mozziconacci Hôpital de Bicêtre (Seine)
(2) Service J Decourt Hôpital de la Pitié Paris
(3) See text
(4) Acid hydrolysis Zimmermann chromogen
(5) Enzymic hydrolysis Porter Silber method
(6) 24-hour pH 1 hydrolysis, quantitative paper chromatographic method
Normal cortisol 20–150 µg cortisone 5–50 µg and aldosterone 0 5–6 µg /24 hours

(Table I) It is interesting to compare their histological aspects the aldosterone producing tumour is very unusual and somewhat glomerular like Of course the hypertension and hyperaldosteronism are not necessarily connected another hormonal production may be implicated

Querido Dr Neher s lung carcinoma results are fascinating We also have seen the combination of lung carcinoma metastasing to the adrenal cortex and hypercorticism like Cushing s syndrome (Querido A (1955) *Ned Tijdschr Geneesk* 99, 43) We thought that it might be rather more than a coincidence because it had been maintained that the incidence of carcinoma in patients with Cushing s disease is greater than normal However if I understood Dr Neher well he was thinking in terms of destruction of the adrenal acting as a stimulus for the other side or for the adrenal itself to make other steroids The extreme rarity of the syndrome compared with the frequency of adrenal metastases in lung carcinoma makes this difficult for me to conceive You might see it in 50 per cent of

the cases and this destruction might go so far that you might have Addison s disease complicating lung carcinoma before the patient dies of his lung carcinoma Now why should it happen so rarely that the reaction of the adrenal causes a Cushing s syndrome?

Neher This is the only case which we have examined and since the patient was hospitalized in Geneva Dr Muller can tell you more about it.

Muller We had the good fortune to study this patient while she was alive Clinically she presented pneumonia and the blood analyses revealed a severe hypokalaemic hypochloraemic alkalosis. At the beginning we were somewhat puzzled by this finding But as we later made the diagnosis of cancer of the lung we thought that this could be one of those rare cases of hypercorticism associated with carcinoma of the lung and metastases to the adrenal gland The elevated plasma corticoids and the high urinary output of 17 hydroxycorticoids confirmed this hypothesis but to our great surprise the urinary aldosterone was low Then without any causal therapy the hypernatraemia (152 m-equiv /l) as well as the hypokalaemic alkalosis disappeared and four days later the patient died

We think that this patient s typical syndrome of hypokalaemic hypochloraemic alkalosis was exclusively due to the increased production of the glucocorticoids and not to aldosterone We take an opposite view to that of Spaulding and Conn who consider these cases as primary hyperaldosteronism of the mixed type However they did not determine the urinary aldosterone All the cases published in the literature so far are very similar to ours but in none has the aldosterone been measured The fact that the full clinical picture of Cushing s syndrome is lacking is not surprising since the evolution in these cases is so rapid (see note p 28)

Mach We had the opportunity to study another identical patient a few years ago (1956 *Helv med acta* 23 301) Fig 1 illustrates the metabolic

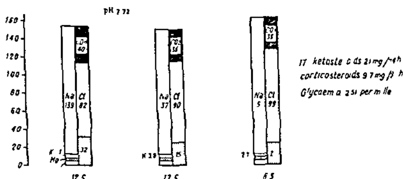


FIG 1 (Mach) Ionograms of a patient with bronchiogenic carcinoma

Note on different occasions the hypokalaemia, hypochloraemia hypernatraemia and alkalosis. The blood sugar is also high as well as the 17 ketosteroid and the corticosteroids in the urine

disturbances Unfortunately aldosterone had not yet been discovered In addition to the hypokalaemic hypochloraemic alkalosis this patient had diabetes mellitus also

Stanbury To mention another aspect of bronchial carcinoma and its relation to adrenocortical function my colleagues Drs Lloyd and

Venning have been impressed by the not infrequent occurrence of low levels of serum sodium in patients with bronchial carcinoma. In two patients studied by them there was an apparent renal salt wasting syndrome. In these patients Dr A. H. Gowenlock found the urinary output of aldosterone to be negligibly small whereas the output of cortisol was normal or slightly increased. One wonders how the presence of a bronchial neoplasm was influencing adrenocortical function in these patients.

Garrod: Concerning Dr Muller's question of severe hypokalaemic alkalosis arising without excessive aldosterone secretion, we have measured the urinary steroid excretions in two of Dr T. M. Chalmers' cases of Cushing's syndrome due to adrenal carcinoma (Garrod, O. (1957). *Proc. roy. Soc. B*, 50, 770). Both had very severe hypokalaemia with very low total body potassium and alkalosis, and in both the cortisol excretion was very high, but with aldosterone excretion at the lowest range of normal. One of these patients also had a raised corticosterone excretion, and after operation the cortisol and corticosterone excretions came down to normal with the aldosterone excretion rising towards the upper normal range. However, during the period of severe hypokalaemic alkalosis the aldosterone excretion was right at the lowest level of normal.

Gabrilove: A number of years ago when Dr Soffer and I and our associates were studying the salt tolerance test in Cushing's syndrome, in only one of a large group of control subjects did we encounter the paradoxical sodium diuresis which is so common in patients with Cushing's syndrome. This occurred in a patient with carcinoma of the lung. We did not have a postmortem examination.

Concerning Dr Neher's interesting report on the varying proportions of adrenocortical steroids that one may find in adrenocortical tumours, I think these observations are particularly important to all of us who have been attempting to correlate the histology of these adrenal tumours with the clinical picture. We had hoped thereby to find that certain histological types of tumours are associated with the elaboration of particular adrenocortical steroids and, as a corollary, to deduce the relation of the zones of the adrenal to the biosynthesis of these compounds. In view of the data presented, it is unlikely that we will be able to solve the problem solely in this fashion, although the tumours that Dr Baulieu has discussed appeared to me to resemble the zona glomerulosa. His demonstration of essentially adrenogenital clinical manifestations in association with increased excretion of aldosterone and glycogenic corticoids emphasizes an important point. Particularly in patients with malignant adrenocortical tumours there may be noted a mixture of the clinical manifestations of the various syndromes of hyperfunction of the adrenal cortex or at least concomitant excessive urinary excretion of aldosterone, the glycogenic corticoids or the sexogens in varying combinations. I think these presentations and this viewpoint emphasize the need for caution in the correlation of the histological appearance of the tumour and the clinical disorder. They also help explain the deduction of Conn that the zona fasciculata rather than the zona glomerulosa was concerned with the elaboration of aldosterone, since in his original case he noted atrophy of the zona fasciculata of the adrenal contralateral to the resected tumour.

NOTE ADDED IN PROOF. *Muller*: Recently in another patient with hypokalaemic, hypochloraemic alkalosis and bronchial [illegible] ma 17-ketosteroid and 17-hydroxycorticoid were again [illegible] and aldosterone excretion was at the lowest level of normal. Autopsy confirmed the bronchial carcinoma but microscopically no metastasis to the adrenal gland could be found (Leaf and Muller, to be published).

COMPARISON OF THE EFFECTS OF ALDOSTERONE CORTEXONE AND CORTISOL ON ADRENALECTOMIZED RATS UNDER VARIOUS SALT LOADS

P Desaulles

Research Laboratories of the Pharmaceutical Department CIBA Limited Basle

IN previous studies we have shown that adrenal steroids may act on different functional systems These actions are often as important as those which are regarded as specific (Meier and Desaulles 1956 1957*b*) Modification of homeostasis in experimental animals by changing the intake of water and electrolytes greatly affects their response to treatment with adrenal steroids (Meier and Desaulles 1956 Selye 1943 Toussaint 1951)

In order to shed further light on certain pharmacological effects of aldosterone on water and salt metabolism as well as on connective tissue reactions we have compared its effects in animals submitted to various loads of water and salt together with those of cortexone and cortisol

METHODS

The effects of steroids on urinary and electrolyte excretion were tested by the methods described in detail elsewhere (see Desaulles and Meier 1956) The substances were given in oily solution over a wide range of dosages Aldosterone was given in doses ranging from 1 to 100 μg /kg cortexone from 15 to 500 μg /kg and cortisol from 200 μg /kg to 25 mg /kg

Connective tissue reactions were tested by means of the foreign body granuloma technique using cotton pellets (see Meier Schuler and Desaulles 1950) The weight of the granuloma was determined on the seventh day following implantation All the substances studied were given subcutaneously in oily solution Aldosterone was given in doses ranging from 0 3 to 5 mg /kg cortexone from 1 to 25 mg /kg and cortisol from 1 to 50 mg /kg Male rats were used for all these experiments The experimental animals weighing between 120 and 150 g were housed in metal cages at a constant

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change although its concentration diminishes With a load of hypertonic saline solution marked diuresis occurs together with a very high increase in both the excretion and concentration of sodium On the other hand the quantity of potassium excreted remains practically constant only its concentration diminishing

These different forms of loads yield different patterns of excretion which it is important to recognize before examining the effects of the steroids (Fig 2) As regards the urinary output in response to different forms of water or salt loads we observe that aldosterone greatly enhances the impaired diuretic response of animals submitted to a

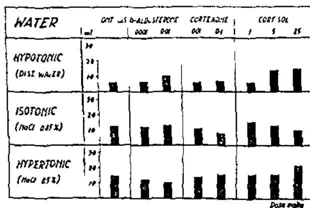


Fig 2 Urinary volume excretion of adrenalectomized male rats submitted to different forms of load and treated with aldosterone cortexone or cortisol

The abscissa hows doses given in mg /kg. subcutaneously The ordinate shows urinary exc etion 2 ho rs in ml compared w th the control gro p

water load under increasing salt loads aldosterone does not modify the diuretic response of the animals while under hypertonic salt loads it actually induces marked water retention

On animals submitted to a water load cortisol has effects that greatly resemble those of aldosterone Cortisol is less active but its effects are qualitatively more intense With increasing salt loads the relative diuretic response diminishes consistently Cortexone has a different pattern of activity It does not modify the impaired urinary excretion of water loaded animals It produces a definite urinary retention under an isotonic salt load and induces slight diuresis under hypertonic salt loads

The steroids studied have marked effects on the sodium excretion pattern under these experimental conditions Under a water load

relative humidity of 75 per cent, the animals being kept either isolated (electrolyte and urinary excretion) or in groups of 6 (granuloma)

The animals on a salt free diet were given a fat casein and carbohydrate diet containing less than 0 01 per cent NaCl Those on a normal diet were given full standard rat cake (Wayne Lab-Blox Rat Diet Allied Mills Inc Chicago) and water *ad libitum* The animals on a high salt diet were given full standard rat cake and 1 per cent NaCl *ad libitum*

EXPERIMENTAL RESULTS

The use of water loads and loads of NaCl solutions of varied concentrations produces characteristic changes in the excretion

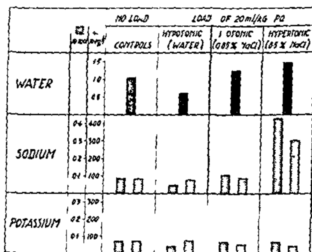


FIG 1 Urinary volume and electrolyte excretion of adrenalectomized male rats used as controls under different forms of loads Compared with unloaded control animals

pattern of urine and electrolytes in control animals on a normal diet (Fig 1) Following a water load there is a very sharp decrease in the urinary output during the time of observation The sodium and potassium excretion corresponds approximately to the changes in the quantity of urine the concentration of electrolytes remaining the same as in the controls Under a load of isotonic saline solution a great increase occurs both in the urinary output and in the sodium excretion The concentration of sodium in the urine rises only slightly The quantity of potassium excreted undergoes hardly any

Aldosterone has under different forms of loads, an enhancing effect on the potassium excretion (Fig 4) the intensity of this effect shows an inverse relationship to the sodium pattern Under a water load aldosterone produces a marked stimulation of the impaired potassium excretion under salt loads of increasing concentrations the stimulated potassium excretion diminishes rapidly Cortisol produces effects that are comparable to those of aldosterone but of much greater intensity In both cases the concentrations of potas sium in the urine show the same pattern under different loads Cortexone exerts somewhat different effects Under a water load

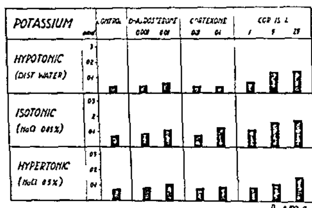


Fig 4 Potassium excretion of adrenalectomized male rats under different forms of load and treated with aldosterone cortexone or cortisol

Ab cissa and o dinate as n F g 3

cortexone in the doses studied does not modify the impaired potassium excretion Under an isotonic solution load which produces marked water retention together with sodium retention the excretion of potassium is very high but under hypertonic saline loads which are followed by slight sodium retention with high doses of cortexone the potassium excretion shows only very mild stimulation Only under physiological saline loads does cortexone induce a marked rise in the potassium concentration of the urine In the case of water or hypertonic saline loads there are only minor changes in concentra tion

If on the other hand we study the intensity of effect of different doses of aldosterone cortexone and cortisol on the formation of the foreign body granuloma in animals submitted to diets containing

aldosterone exerts a very pronounced sodium retaining action which constantly diminishes in intensity as the salt load is increased (Fig 3) Cortexone too produces a more marked sodium retention under a water load but when the salt load is further increased, the retentive effect of cortexone diminishes more rapidly than in the case of aldosterone the result being that under hypertonic saline loads there is practically no retention at all In this respect cortexone is not only less active quantitatively than aldosterone but it does not have the same quality of effect as regards its capacity to retain sodium

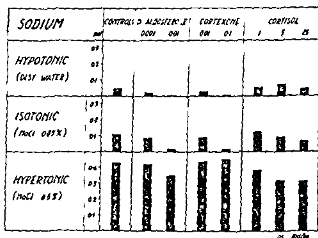


Fig 3 Sodium excretion of adrenalectomized male rats under different forms of load and treated with aldosterone cortexone or cortisol

Abscissa as in Fig. 2

Ordinate Sodium excr t on in 2 hours in mM compared w th contr ls

under different forms of loads With cortisol we have a completely different type of effect Small doses of cortisol tend to produce sodium excretion and in this respect the sodium excreting activity of cortisol is much more marked under a water load than under salt loads where the effect is less marked or even absent High doses of cortisol produce a tendency to retain sodium Under a water load this effect is only slight under isotonic salt loads it tends to become increasingly marked while under hypertonic salt loads it is very high Under cortisol treatment the sodium concentration in the urine shows a pattern analogous to that of the absolute values under different loads

but completely different from those of cortexone Weight for weight, aldosterone is much more potent than cortisol its effect however is qualitatively different, and under salt loads it may disappear altogether

This finding that, even in short term tests aldosterone can restore to normal the greatly impaired urinary output of adrenalectomized rats after a water load confirms the observations of Renzi and co workers (1956) on water intoxication in rats where aldosterone was found to act qualitatively like cortisol with cortexone in the same experiment exerting little or no activity These findings are also in agreement with the observations of Gross and Dettbarn (1956) and Gross (1956) concerning the effects of cortisol and cortexone in adrenalectomized dogs but they differ completely regarding the effects of aldosterone

The urinary output of animals receiving salt loads is clearly increased a fact which can be related to the hypothesis that NaCl partially normalizes the greater sensitivity of adrenalectomized animals to antidiuretic hormones Cortisol further enhances this normalizing effect, its diuretic action being however relatively less pronounced with increasing salt loads (Gaunt, 1951 O Connor 1955)

As regards the response to a salt load the principal action of aldosterone seems to be that it maintains an equilibrium between sodium retained and fluid volume urinary excretion varying in inverse proportion to sodium retention

In the presence of isotonic loads cortexone has a clearcut antidiuretic action which disappears under hypertonic loads and this effect too might well be attributable to a qualitative difference between the potent sodium retaining properties of cortexone and its relatively weak antagonistic effect on the sensitivity of experimental animals to antidiuretic hormones (Garrod Davies and Cahill 1955)

The effect of aldosterone on sodium excretion differs not only *quantitatively from that of cortexone but also qualitatively* from that of cortexone and of cortisol Aldosterone differs from cortexone by virtue of the qualitative differences in its sodium retaining properties under a water load aldosterone in this case being about 100-fold more active than cortexone under similar conditions (Cortexone has on the other hand the ability to induce diuresis with low concentrations of sodium in much smaller relative doses than aldosterone)

different amounts of salt, it is possible to make further differentiations (Fig 5)

Cortexone in doses of from 1 to 25 mg/kg subcutaneously and aldosterone in doses of from 300 µg/kg to 5 mg/kg subcutaneously enhance foreign body granuloma formation when administered systemically An increase in the quantity of salt in the diet provokes a further rise in the weight of the granuloma Cortexone shows this effect particularly clearly Cortisol in a dosage ranging from 0 5 to 50 mg/kg subcutaneously, inhibits foreign body granuloma formation when administered systemically In response to an increase in

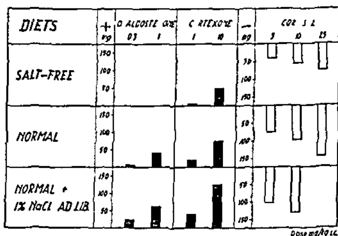


Fig 5 Role of dietary salt in the stimulatory or inhibitory effect of aldosterone cortexone or cortisol on foreign body granuloma formation in adrenalectomized male rats

Abscissa as in Fig. 2.

Ordinate D ff re e between f esh w ight of th granuloma (in mg) in experimental animals and controls. In h case th animals were of the same w ght.

the salt content of the diet there is a marked increase in the intensity of this effect so that a high salt diet enhances both the stimulating effects of aldosterone and cortexone as well as the inhibiting effect of cortisol on foreign body granuloma formation

DISCUSSION

With the aid of different forms of water and salt loads it is possible to characterize certain aspects of the action of aldosterone and to compare them with the effects of cortisol and cortexone

As regards water excretion in adrenalectomized rats aldosterone has effects on urinary output that are similar to those of cortisol

To produce effects on mesenchymal reactions the doses of aldo sterone must be about 200 times greater than those necessary for an effect on electrolytes For cortexone the doses needed for connective tissue reactions must be about 50 times greater than those producing effects on electrolytes On the other hand in the case of cortisol the effects on connective tissue and on electrolytes can be elicited with approximately the same dose

The relatively very high dosages of both cortexone and aldosterone necessary to produce modification of the connective tissue reaction compared with cortisol leads to the question of the specificity of the systemic effects of those substances on the connective tissue With cortexone certain discrepancies are apparent as between local and systemic effects and between systemic effects under certain conditions which we have already noted (Meier *et al* 1952 Meier and Desaulles 1957*a*) On the other hand recent studies by Lehr and by Selye tend to show that cortexone may act only in a secondary capacity (Lehr Cheng and Milors 1954 Selye and Bois 1957)

The available data do not permit comprehensive conclusions to be drawn concerning the analogy between aldosterone and cortexone in this respect The problem still awaits clarification On the other hand the correspondence between local and systemic effects of cortisol on connective tissue reactions tends to prove their specificity

The limited work done to date is too restricted to ascertain the effects of aldosterone Certain conclusions can however be drawn As regards effects on electrolyte metabolism urinary excretion and connective tissue reactions there are important qualitative as well as quantitative differences between cortexone and aldosterone In certain situations aldosterone effects can mimic those of cortisol so that it has a position of its own However, the specificity of certain of its actions remains to be definitely proved Before the physiological role of aldosterone can be fully defined still more investigations are necessary

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Cortisol differs from aldosterone in that it causes a rise in sodium retention in response to increasing NaCl loads The fact that cortisol may induce sodium retention in the rat is in line with the experimental results of other investigators (Johnson 1954 Dorfman 1949 Kagawa and van Asman 1957) The fact that increasing NaCl loads enhance this retention may be traced to the observation that cortisol produces in adrenalectomized animals increased sodium filtration as well as a rise in the amount of sodium absorbed by the tubules (Davis and Howell 1953) and that these effects depend on the one hand on the degree to which the effects of antidiuretic hormones are normalized and on the other hand on the dose used (Liddle Pechet and Bartter 1954 Gaunt, Birnie and Eversole 1949)

Cortexone seems to have effects on potassium excretion somewhat comparable to those of aldosterone The amount of potassium excreted is directly correlated to the sodium retention this being particularly clear in the case of aldosterone These findings seem to tally with clinical observations (Buchborn Koczorek and Wolff 1957)

The potassium excreting action of cortisol is correlated with its catabolic effects The diminution in the relative potassium excretion under increased salt loads as we and others (Kagawa and van Asman 1957) *have found is due to the fact that potassium excretion is very rapid and that under increasing salt loads the* maximum of excretion appears before the collecting time If we compare the doses effective on electrolyte metabolism with those having an effect on connective tissue it is possible to make further differentiations Aldosterone has an optimal dose effect range of about 0 5 to 25 μg /kg on electrolyte metabolism in rats According to results published by us and by other authors (Selye 1955 Gaunt 1957 Meier Desaulles and Schar 1956) it produces effects on connective tissue formation only in doses ranging from about 300 μg /kg to 5 mg /kg i e it is relatively much less potent in this capacity than cortexone its active dose range being of the same order

Cortexone has a dose effect range of 12 5 to 500 μg /kg on electrolyte metabolism in rats Its effects on connective tissue formation are most marked in a dose range of 1 to 25 mg /kg For cortisol these values are respectively 0 5 to 25 mg /kg for electrolytes and about the same for connective tissue reactions

FURTHER EVIDENCE FOR A QUALITATIVE DIFFERENCE BETWEEN ALDOSTERONE AND CORTEXONE

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SINCE aldosterone has been isolated and has been found to be quantitatively about 25 times superior to cortexone as regards sodium retention one of the most interesting problems has been to discover qualitative differences between these two cortical steroids In their first papers Simpson and Tait had already shown that potassium excretion is not enhanced to the same degree as sodium retention (Simpson and Tait 1952 Tait Simpson and Grundy 1952) and this has been confirmed by Desaulles Tripod and Schuler (1953) In the adrenalectomized dog we have found a slight loss of sodium and water when changing from a maintenance dose of cortexone to one of aldosterone and simultaneously there was a slight increase in the haematocrit value which in general is lower in cortexone treated animals indicating a certain degree of hydraemia Furthermore there was no increase in plasma sodium concentration when about 3–5 times the maintenance dose of aldosterone was administered to the adrenalectomized dog Due to shortage of the hormone at that time these high doses could only be maintained for a few days In acute experiments in normal and adrenalectomized rats Simpson and Tait (1955) however found a close relationship between the dose of aldosterone and the resultant sodium retention In loading experiments with salt and water some differences between dogs treated with cortexone as compared with aldosterone have also been observed (Gross and Dettbarn 1956) In addition Selye (1955) has reported that the inhibiting effect of aldosterone on the anti inflammatory action of cortisol is about the same as that of cortexone i e relatively moderate compared with its sodium retaining activity It has been reported recently that cortexone and aldosterone have different effects in mice on electrolytes in skeletal muscle and in the brain (Woodbury and Koch 1957)

There are thus various findings from which it is obvious that both

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[Discussion of this paper was postponed until after the paper by Drs Gross and Lichtlen.—EDS]

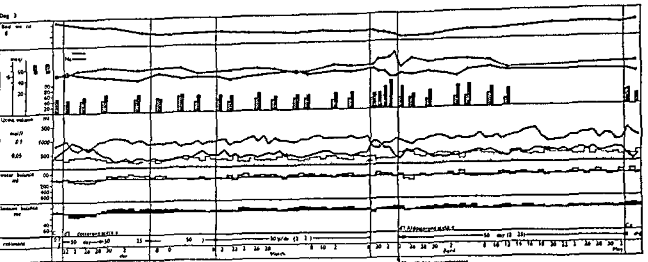


FIG 1 Bilaterally adrenalectomized dog (operation 27 3 56) maintained for three months with daily doses of 0 05 mg DL aldosterone acetate During the first 10 days after changing from daily dose of 0 7 mg cortexone acetate aldosterone was given once daily then in two subdivided doses From the 15th to 28th day of treatment total daily dose of 0 075 mg was given Interruption of treatment from the 56th to 61st day

Read ng f om abo e body weight (kg) plasma sodium and pot s um in m-eq /l haematocrit (▭) n n-protein nitrogen (▭) Urinary volume and r nary sod um and potass u i concentr tions

Water bal nce ml } based on urinary o tput
Sodium balance m-equiv

corticoids do not have the same pattern of activity and a better characterization of the properties by which the two compounds are distinguished from one another is necessary For this purpose we examined aldosterone in two ways

(1) by a quantitative and qualitative comparison with cortexone in the adrenalectomized dog

(2) by overdosage experiments in the dog and in the rat

1 MAINTENANCE DOSE OF DL ALDOSTERONE ACETATE IN THE DOG

In adrenalectomized dogs the daily maintenance dose of DL aldosterone acetate has been found to be about 1 7–2 times greater than that of free D aldosterone The dog may be maintained in a well compensated state for several months with a total daily dose of 50 µg of the racemate in oily solution subdivided in two doses of 25 µg at an interval of 10 hours (Fig 1) If these 50 µg are injected only once during 24 hours compensation is not as complete and symptoms of slight insufficiency may develop When treatment is changed from a daily maintenance dose of 0 7 mg cortexone in oily solution to 50 µg aldosterone there occurs a negative sodium and water balance and simultaneously a decrease in body weight Potassium concentration in the urine also declines Plasma sodium diminishes transitorily while the plasma concentration of potassium rises somewhat The non protein nitrogen concentration increases too whereas the haematocrit value does not change correspondingly These findings are in agreement with our results published earlier (Gross and Gysel 1954) Raising the daily dose to 75 µg (50 + 25) leads to a positive sodium and normal water balance The plasma concentration of sodium however may still decrease while the concentration of potassium increases Plasma concentration of both electrolytes returns to normal values only after a few days of treatment The same happens with the non protein nitrogen concentration There is therefore a certain delay in the response to an increase in the dose of aldosterone (Fig 1) Interruption of treatment leads to sodium and water loss rapid decrease in plasma sodium and steep increase in plasma potassium and non protein nitrogen Treatment of the insufficient animal with aldosterone is followed by an immediate retention of sodium and the sodium balance remains positive for the following two weeks

FIG 2 *a–d* Development of insufficiency symptoms in an adrenalectomized dog after interruption of treatment.

(*a*) Treatment with DL aldosterone acetate before and after interruption
(*b*) Treatment with DL aldosterone acetate before and with cortexone acetate after interruption
(*c*) Treatment with cortexone acetate before and after interruption
(*d*) Same sequence as (*c*) in another animal

For treatment of severe symptoms of insufficiency cortexone glucoside (45 mg i.v.) and cortisone (25 mg s.c.) have been administered in each experiment

Reading from above: body weight (kg), plasma sodium and potassium in m-eq/l, haematocrit (▨), plasma non-protein nitrogen (▭). Urinary volume and urinary sodium and potassium concentrations.

Water balance ml }
Sodium balance m-equiv } based on urinary output

The longest duration of DL aldosterone acetate treatment in an adrenalectomized dog is now three months with an average daily maintenance dose of 50 µg (Fig 1) The animal was in excellent condition during the whole period of the experiment with the exception of the five days when treatment had been stopped

There is a marked difference in the sodium and water excretion as well as in the variations of the plasma electrolytes when treatment is interrupted during cortexone or during aldosterone therapy The following sequences of treatment are possible

(1) Cortexone ⟶ interruption ⟶ cortexone
(2) Cortexone ⟶ interruption ⟶ aldosterone
(3) Aldosterone ⟶ interruption ⟶ cortexone
(4) Aldosterone ⟶ interruption ⟶ aldosterone

If treatment with cortexone (0 7 mg daily) is stopped a marked excretion of sodium and water occurs together with a sharp decrease in body weight The amounts of sodium eliminated in the urine decline and after 3–4 days an equilibrium between sodium intake and excretion is attained This coincides with the occurrence of severe symptoms of insufficiency and a slight increase in the excretion of potassium The haematocrit and the non protein nitrogen level increase markedly as does the plasma potassium concentration whereas the sodium concentration falls Resumption of treatment with the same dose of 0 7 mg cortexone leads to a positive sodium balance due to marked retention of sodium while there is not a correspondingly high excretion of potassium (Fig 2 *c* and *d*)

If treatment with aldosterone is interrupted negative sodium and water balance is less pronounced also the initial loss of body weight (Fig 2 *a* and *b*) The haematocrit increases less markedly while the non protein nitrogen reaches very high values The plasma potassium concentration increases definitely more than after cortexone withdrawal while plasma sodium concentration shows about the same decrease A severe state of insufficiency develops more rapidly than after interruption of cortexone treatment and after 3–4 days treatment has to be re established whereas following withdrawal of cortexone it is possible to wait for 5–6 days When aldosterone is again given the sodium becomes equal but contrary to what happens with cortexone there is a marked excretion of potassium about twice as much as during the aldosterone free period (Fig 2 *a*) If instead of aldosterone cortexone is given to combat the insufficiency

the body which would also explain its shorter duration of action, but this alone does not give a sufficient explanation for various differences in the type of action of the two corticoids The more pronounced water and sodium retention under the influence of cortexone which gives rise to an increase in plasma volume and probably also

FIG 3 Bilaterally adrenalectomized dog (operation 27 3 56) treated with 0 05 mg DL aldosterone acetate daily (subdivided in two doses of 0 0'5 mg.) Increasing the dose up to 1 mg. daily provokes only a slight positive water and sodium balance

R d ng f om bo body w ght (kg) plasma sod m nd potass um n m-equ l haemato-t (▭) n n protein nitr g n (▭) U nary l me and urinary sod um and potass um centr t ons

W t r b lance ml
S d um balance m-eq } based on urin ry o tput

in total extracellular fluid volume as well as the greater loss of potassium cannot be explained by its longer duration of action alone but shows that there is a qualitative difference in its pattern of activity This finds further confirmation in the results of our experiments on aldosterone overdosage in rats

(ii) ALDOSTERONE OVERDOSAGE IN RATS

In addition to a previous experiment with daily doses of 40 µg D aldosterone in rats during 4 weeks which did not reveal any

symptoms the excretion of potassium on the first day of treatment is even more pronounced and simultaneously the sodium retention is more marked As a result of the strong retention of sodium the concentration of sodium in the urine falls to very low levels (Fig 2*b*) The more pronounced excretion of potassium in animals which have been pre treated with aldosterone instead of cortexone occurs whether or not aldosterone or cortexone is given following the state of insufficiency A possible explanation for this high potassium excretion is the lower degree of potassium loss not only during treatment with aldosterone but also after interruption of treatment as compared with cortexone Therefore it may be assumed that in animals which have received aldosterone there is more potassium available for elimination by exchange with reabsorbed sodium than in cortexone treated animals The more rapid increase in plasma potassium concentration in aldosterone treated animals after interruption of treatment also gives a hint in this direction

2 (i) OVERDOSAGE OF ALDOSTERONE IN THE ADRENALECTOMIZED DOG

If the daily maintenance dose of 50 µg DL aldosterone acetate is increased up to 1000 µg only a slight sodium retention occurs which is definitely less marked than with 700 µg cortexone acetate per day There is no definite increase in the plasma sodium concentration and no decrease in plasma potassium and also the haematocrit value remains constant The non protein nitrogen value is still slightly higher than under cortexone (Fig 3) If treatment with this aldosterone dosage is continued for 3 weeks no symptoms similar to those with cortexone overdosage occur especially no diabetes insipidus like syndrome which may be observed only a few days after the onset of treatment with high doses of cortexone

Although it is too early to draw definite conclusions from these preliminary observations with aldosterone overdosage it is evident that the natural sodium retaining hormone does not produce the same degree of pathological sodium retention as may be produced with comparable or even with lower doses of cortexone The danger of producing overdosage reactions with aldosterone is definitely less than with cortexone especially in the adrenalectomized animal which is more sensitive to cortexone overdosage than is the intact animal This better therapeutic range of aldosterone may be due partly to a more rapid enzymic degradation of the hormone in

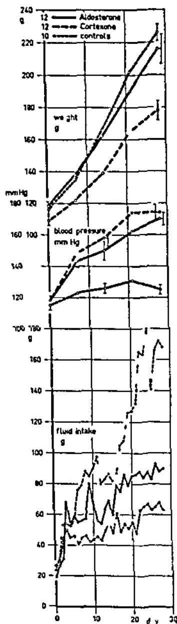


FIG 4a Development of hypertension during overdosage of DL aldosterone acetate (0 5 mg. daily) and cortexone acetate (2 5 mg daily)

Reading f m bo e w gh in g. blood pressure in mm. Hg, fluid ptake n ml.

All animals w re un l terally nephrectomized and had 1 / sal ne as drinking fluid.

hypertensive action of the hormone (Gross, Loustalot and Meier 1955), two further experiments with 250 and 500 µg of DL aldosterone acetate per day have been performed With the lower of these two dosages out of 10 animals only 2 showed an increase in blood pressure up to 160 mm Hg during the 4-week period of treatment while in the other 8 animals blood pressure did not exceed values of 120–130 mm Hg The consumption of drinking fluid (1 per cent NaCl solution) increased only in the 2 animals which had a definitely elevated blood pressure but was not higher in the other animals than in control animals With 500 µg however the fluid intake increased from an average of 20 ml at the beginning up to a daily amount of 90 ml within 4 weeks while it reached nearly twice this amount in cortexone treated animals The blood pressure rose to an average of 175 mm Hg within this period and was finally as high as in the group of animals treated with cortexone Whereas aldosterone overdosed animals gained weight at the same speed as the control group those overdosed with cortexone had a lower rate of increase in body weight (Fig 4 *a*) In other experiments we have found that there is an inverse relationship between sodium retention and the content of hypertensive substances in the kidney probably mainly renin Estimating the pressor activity of kidney extracts using nephrectomized rats according to a method described previously (Gross and Sulser 1956) we found a diminution after 4 weeks daily treatment with 250 µg of aldosterone and with 500 µg daily the pressor substances disappeared almost completely as also occurred with the kidneys of animals treated with cortexone (Fig 4 *b*) How ever the kidneys did not show macroscopically the same yellowish mottled surface as the kidneys of cortexone treated animals and neither were they as large

From our experiments it is evident that it is possible to induce hypertension with aldosterone if sufficient salt is given simultaneously but that the necessary doses are relatively higher than those of cor texone It cannot be decided yet whether aldosterone overdosage in rats can induce the same degree of renal and vascular damage as cortexone does and if there is a comparable type of proliferative inflammatory lesion such as occurs under high doses of the latter Further experiments especially with long acting forms of aldosterone (crystals various esters) are necessary in order to complete these studies From the results already available however it is possible to conclude that under the conditions of our experiments relatively

with *Addison s disease will be less sensitive to aldosterone than to* cortexone From our present experiments in dogs we may conclude that sodium reabsorption is enhanced under increasing doses of aldosterone but only to a limited degree and that—at least in a *certain dosage range—there is not a corresponding elimination of* potassium as occurs under overdosage with cortexone The only evidence for a pronounced potassium loss under the influence of high doses of aldosterone is the clinical syndrome of Conn with its *typical hypokalaemia and alkalosis There is however some* suspicion that the so called primary hyperaldosteronism described by Conn is not only the consequence of a prolonged overproduction of aldosterone but probably also of other corticoids In tumours *from these patients not only higher amounts of aldosterone but* also of corticosterone have been found (Neher 1956) and on the other hand the excretion of aldosterone in the urine of patients with Conn s syndrome is not always markedly increased and may even *be lower than in patients with secondary aldosteronism who do not* develop a similar degree of hypokalaemia

In summarizing our findings we can state that

(1) adrenalectomized dogs can be maintained in sodium and *water balance during more than three months with doses of* 50 μg DL aldosterone acetate per day

(2) doses of up to 1000 μg administered daily for three weeks do not produce marked sodium retention and do not lead to a diabetes *insipidus like state such as occurs with corresponding overdosage* with cortexone

(3) in the unilaterally nephrectomized rat having 1 per cent saline as drinking fluid an increase in fluid intake and rise in blood pressure develop *but relatively higher doses than those of cortexone* are necessary to obtain comparable alterations

(4) the safety margin of aldosterone is definitely higher than that of cortexone especially in the adrenalectomized animal

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higher doses of aldosterone than of cortexone are necessary to produce an increase in blood pressure These observations do not correspond with those published by Kumar and collaborators (Kumar Anderson and Gornall 1956 Kumar *et al* 1957) who reported hypertension during prolonged treatment with very low doses of aldosterone (0 5–1 0 μg every two days) These findings

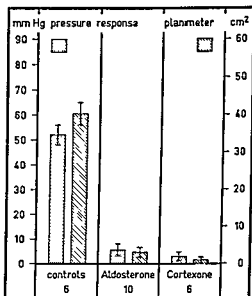


FIG 4 *b* Pressor activity of fresh kidney extracts tested in the nephrectomized rat The kidneys were removed from the animals at the end of the experiment shown in Fig 4 *a*

F m l ft to ght o t l ld t one-tr ted (0 5 mg daily) and co t on tr ted (2 5 mg daily) animals

Dotted lum m xim l inc ease of bl od pr ssure after injection f th kidn y extract in mm Hg

H t hed ol mns int g al of blood p essure curve in cm

however have not been confirmed by Gaunt Ulsamer and Chart (1957)

The observations made in dogs and rats demonstrate that these two species of animals are less sensitive to overdosage with aldo sterone than to high doses of cortexone In order to produce typical alterations due to excess sodium retention relatively higher doses (as regards sodium retaining capacity) of aldosterone than of cortexone have to be given Therefore it is probable that patients

Bartter Dr Gross were the dogs and the rats which you and Dr ;saulles have described force fed so that you could be sure of a constant dium intake throughout the studies?

Gross In the studies I mentioned there was a constant sodium intake e rats were not force-fed

Bartter To what extent do you think that the differences between the tion of aldosterone and cortexone are explainable by differences in sorption or destruction?

Desaulles We have found that only under certain conditions especially ider fluid loads there is in the adrenalectomized rat a clearcut difference :tween cortexone and aldosterone in so far as it is impossible to get with ortexone a normalization of the impaired water excretion whereas with dosterone it is always possible to do so I don t know if this effect is roduced by an accelerated metabolic destruction of the product but it is npossible to get it even if you increase the dosage of cortexone. On the ther hand, if you use a higher salt load you obtain a diuretic response to ortexone but this does not happen with an isotonic load This observa on corroborates Dr Gross findings on the dog which shows under high alt intake a diuretic response to cortexone

As regards Dr Vesin s point we have carried out some water load xperiments on normal rats Because of the size of the animal it is possible o give very high doses of aldosterone Under these conditions we do get a learcut diuretic response with aldosterone but this response is very slight compared with the response to cortisone cortisol and particularly pred usone The latter has in this respect, a very marked action

Luft Some years ago (Luft *et al* (1954) *Recent Progr Hormone Res* 10 425) we performed metabolic studies in normal subjects given 20 mg. of cortexone daily for several weeks We found that in these subjects there occurred an increase in the exchangeable sodium which could not be accounted for by the balance The increase amounted to several hundred m-equiv of sodium. These findings were interpreted as demonstrating a mobilization of previously non-exchangeable sodium of the skeleton by the prolonged cortexone administration There was a simultaneous increase in the urinary output of calcium It is certainly of great interest to know whether aldosterone is able to induce similar metabolic changes Has Dr Gross any experience along these lines?

Gross We have no experience in this direction but it would be of interest to repeat those experiments with aldosterone

Garrod We should remember that the action of the glucocorticoids on water diuresis is a very complicated one! There are undoubted tubular effects then there is the effect on glomerular filtration rate and when you come to pharmacological dosages as Rosenbaum and co-workers (Rosen baum, J D Davis R K and Ferguson B C (1952) *Proc 43rd meeting Amer Soc clin Invest* p 47) showed some years ago in man and Davis and Howell (Davis J O and Howell D S (1953) *Endocrinology* **52**, 245) in the dog you may even double the normal glomerular filtration rate which must greatly increase the amount of water being delivered to the distal tubules Also I have found occasionally that in adrenal insufficiency one cannot correct the diuresis by giving cortisone if the patients are sodium depleted but after having corrected the sodium depletion by giving cortexone then by giving cortisone one can obtain a normal water diuresis

Morel I think the action of aldosterone and cortexone on the excretion

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DISCUSSION

Vesin I would like to know whether aldosterone is capable of correcting the delayed water diuresis in adrenal insufficiency and whether it has any effect on water elimination in normal people and animals We are interested in this aspect because of the well known diuretic effect of cortisone not only in adrenal insufficiency but also in normal subjects and especially in oedematous patients We have been interested in this diuretic effect of cortisone and more recently of prednisone in patients with oedematous cirrhosis and other types of oedema We have carried out in these patients water loading tests similar to those described by Dr Soffer and we found that in some of them cortisone and especially prednisone did correct the delayed water diuresis Since this effect of cortisone on the tubular reabsorption of water manifests itself not only in adrenal insufficiency but also in normal and oedematous patients we think that we are dealing here with a pharmacodynamic action of cortisone and prednisone In our opinion it lowers the value of such tests in the diagnosis of adrenal insufficiency

Gross We carried out some experiments with aldosterone and water load in the normal dog but with doses of up to 0 2 mg per day we could not find an influence on fluid excretion after water load With cortisone or prednisone however we could accelerate fluid excretion after water load especially in the first 4-hour period The amount of water we give is 90 ml /kg In the adrenalectomized dog maintained with aldosterone as we have published fluid elimination after water load is not normalized and is not better than in the cortexone treated animal This has been confirmed in the human by Prof Mach and his collaborators Even with high doses of aldosterone we never attained a normalization of water excretion in loading experiments

Mach Dr Fabre in my Clinic showed that you cannot normalize the water load test with aldosterone in an Addisonian patient with doses of 200 µg of aldosterone per day

Gabrilove We can confirm the finding of Prof Mach With 100 µg of aldosterone we were unable to alter the water excretion in the water load test in one Addisonian patient

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diuresis improved (without being restored to normal) and the excretion of antidiuretic material (which we think is antidiuretic hormone) fell progressively to 16 m unit/24 hours. Treatment with cortexone and cortisone markedly depresses the urinary excretion of antidiuretic factor I wonder if similar experiments have been carried out with aldosterone

Gabrilove In regard to the water load test it has been claimed that alcohol blocks the elaboration of antidiuretic hormone and both Dr Epstein at Yale and subsequently our group have taken Addisonian patients and subjected them to water loading prior to and following the administration of alcohol We have been unable to alter the results of the water tolerance test by the administration of alcohol even in quantities large enough to induce intoxication Indeed some of the patients could not be tested because of a drunken stupor following the ingestion of the alcohol Our conclusions although based on the non specific and rather indirect evidence were that the abnormality in water tolerance of patients with Addison s disease was not due to the elaboration of antidiuretic hormone

In regard to the extrarenal effects of cortexone and cortisone it has been shown at our Institution that the extracellular fluid space as measured by thiosulphate or inulin with the difficulties involved in evaluating such procedures can be altered by the administration of these hormones without altering the external sodium or water balance In patients on a salt free diet or on a measured sodium intake there is an increase in the extracellular space for 8 to 10 days and then there ensues a spontaneous decrease to normal in the size of the space. These alterations in extracellular fluid take place even though any changes noted in the external sodium and chloride and water balance are inadequate to explain them. These data would therefore indicate an extrarenal effect of cortisone and cortexone

Stahl Concerning the interrelationship between the posterior pituitary and the adrenals we have made the following observation bleeding of a normal dog produces about one hour later the appearance of an antidiuretic effect in the urine (as seen in a test dog) If the same dog is bled at the height of polyuria after cortexone and salt such an antidiuretic effect is not seen On cortexone alone without additional salt behaviour in the dog after bleeding is normal These experiments do not suggest an inhibitory effect of cortexone on the posterior pituitary (See also p 167)

Mach In connexion with the data presented by Dr Gross concerning the action of DL aldosterone in the adrenalectomized dog I would like my collaborator Dr E Engel to report on some of our preliminary work on DL aldosterone in the human

Engel These preliminary results were obtained on the effect of DL aldosterone acetate in a 43 year old Addisonian patient The patient was maintained on a constant intake in every respect During the first period this man was receiving 25 mg of cortisone per day Then he received DL aldosterone acetate in two injections per day As Fig 1 shows after a 6-day control period of 25 mg of cortisone per day the subject received DL aldosterone for 6 days The dosage was respectively 600 µg for 2 days then 400 µg and for the last period 300 µg The decreased urinary sodium and chloride excretion during aldosterone therapy is clearly shown in Fig 1 the overall sodium retention during aldosterone therapy amounts to 249 m equiv The urinary potassium elimination was not changed Concomitantly we noticed a decreased urine output and a slight increase in weight The blood pressure however was not modified throughout the

of water is complicated too and I should like to hear Dr Gross opinion on whether the qualitative differences between these two substances may be related to qualitative differences in the action on the kidney tubule itself or to extrarenal factors

Gross Certainly we have thought about that but so far there is no clear experimental evidence to prove any extrarenal effect of cortexone The same problem arises now with aldosterone Dr Bartter have you any idea about this?

Bartter Is there any convincing evidence that cortexone has any action *in the animal which has been prepared previously by vigorous sodium* restriction so that the urinary sodium is virtually zero? This is perhaps the crux of the matter if you want to show an extrarenal action you block the renal tubular action completely by rigorous sodium restriction and any action which you then see must be extrarenal

Luft May I again refer to our studies mentioned above (Luft *et al* 1954 *loc cit*) In two of our studies the subjects were kept on a low sodium diet containing 10 m equiv of sodium per day and after some weeks on this regimen they were given cortexone in a dose of 40 mg daily In these subjects the characteristic findings were no significant change in the external balances of sodium and chloride no change in the exchangeable sodium an increase in the extracellular and a concomitant decrease in the intracellular fluid compartment

Bartter Did you try salt depletion alone observing the patients for the same length of time under the influence of endogenous aldosterone?

Luft Yes during the extended control period on a low sodium diet before administration of cortexone (in one case 4 weeks in a second 7 weeks) there was no change in total exchangeable sodium

de Graeff I am fascinated by the contrast between the very slight hypertensive effect of aldosterone in the experiments of Dr Gross and the clearcut hypertension which can be found in human primary hyperaldosteronism. A few years ago Dr Gross you compared in the rat the hypertensive effect of cortexone and aldosterone in dosages which gave the same retention of sodium Since there have been many publications about an abnormal distribution of sodium between the extracellular and intracellular compartments in human and experimental hypertension do you know whether the distribution of the retained sodium in the aldosterone treated and cortexone treated animals was different?

Gross So far we have not investigated the distribution of sodium in the intra and extracellular compartments under high doses of aldosterone We have compared 40 µg of D aldosterone with 2 5 mg of cortexone acetate and recently we have compared 250 and 500 µg of DL aldosterone acetate with 2 5 mg of cortexone acetate in the rat following in each experiment blood pressure fluid and salt uptake With the 40 and 250 µg there was no increase in blood pressure and no increase in fluid and salt uptake comparable to that in cortexone treated animals

Bastenie Dr Gross have you found depression of the posterior pituitary in relation to the action of steroids on water diuresis?

Gross We did not investigate this

Bastenie We carried out a series of water load experiments in an Addisonian patient and estimated the antidiuretic activity of the urine according to the method of Bellens (Bellens R (1957) *Arch int Pharmacodyn* p 101) Before treatment the amount of antidiuretic material was high (250 m unit/24 hours) Under the influence of cortisone water

than is aldosterone Furthermore cortexone and especially corticosterone have for a given sodium retention a much greater activity on potassium excretion than has aldosterone (Table I) and corticosterone of all the

Table I

Substance	*Dosage necessary to induce a 50% sodium retention (ED_{50}) mg /kg subcutaneously*	*Output for the same dosage*	
		Potassium	*Urine*
Aldosterone	0 0015–0 002	48%	—
Cortexone	0 035–0 05	50–60%	−20%
Corticosterone	8 0	200%	+16%

Experimental conditions identical with those described on p 29 Load used 20 ml /kg. of 0 85 / NaCl solution

steroids having sodium retentive activity is relatively the most potent in its effect on potassium excretion, its effect on potassium excretion being comparable to that of cortisol

The use of water loads containing increasing amounts of NaCl diminishes as under aldosterone or cortexone the sodium retaining properties of corticosterone Despite the fact that under certain conditions corticosterone behaves in a manner similar to that of cortisone and cortisol (e g on glycogen deposition in the liver) these two compounds act in the rat under similar conditions in a completely opposite direction an increasing salt load producing an augmentation of the sodium retaining properties of these compounds instead of a diminution Corticosterone acts on water elimination in the same manner as aldosterone does but it is much less active on a weight basis The use of salt loads of increasing concentrations augments the very intense potassium excretion the effects of corticosterone being in this case comparable to those of cortisol

experiment. When we resumed cortisone the changes were reversed i e urinary sodium and chloride as well as the urine output increased and the weight fell It is interesting that the patient did not feel subjectively so well during *aldosterone treatment as he did on cortisone*

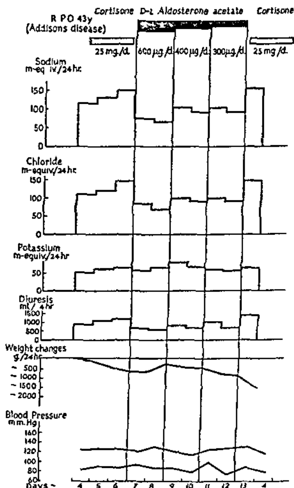


FIG 1 (Engel) Electrolyte excretion diuresis weight changes and blood pressure modifications in a patient with Addison s disease while on synthetic DL aldosterone

Desaulles We have some data on the different effects of corticosterone on electrolyte metabolism in rats compared with those of cortexone and aldosterone Corticosterone has a much less marked activity on sodium retention than has cortexone or aldosterone i e only about 1/200 that of *cortexone* and about 1/4250 that of aldosterone On the other hand it must be remembered that corticosterone is secreted in much greater amount

The second is concerned with a re evaluation of the rôle of the pituitary gland in the secretion of aldosterone

Methods

Beef adrenal glands of freshly killed animals are obtained from the slaughterhouse The glands are dissected free of fat and bisected The medulla is discarded Strips of the adrenal cortex are cut into slices 0 3 to 0 5 mm thick The zona glomerulosa is separated from the inner zones with a razor blade The slices of the glomerulosa and of the two inner zones are incubated separately in Krebs Ringer bicarbonate medium with added glucose (Saffran and Schally 1955) The incubation is carried out in a Dubnoff metabolic shaker-incubator at 38 C in an atmosphere of oxygen 5 per cent CO_2 After the first hour (pre incubation period) the media are poured off and replaced by an equal quantity of the same solution (10 ml /g of tissue) The incubation is allowed to continue for an additional two hours (incubation period) Corticosteroid precursors are always added to the flasks in methanol solution at the beginning of the incubation period The methanol is evaporated under a stream of nitrogen The cortical slices and the Krebs Ringer bicarbonate solution are then added to these flasks When the incubation period is extended to four hours the slices are transferred after two hours to a fresh set of flasks containing the same proportion of precursor and of Krebs Ringer medium At the end of the incubation period the media are decanted and frozen until extraction

The media are extracted first with twice and then with once their volume of redistilled chloroform The chloroform extracts are centrifuged to break down the emulsion filtered into round bottom balloons and evaporated to dryness *in vacuo* at a water bath temperature of 50 C

The separation of the corticosteroids is carried out by means of two chromatographic systems run at 25 ± 2 C The crude chloroform extract is first applied on the benzene aqueous methanol system (Bush 1952) which provides a good separation of cortisol and corticosterone The zone containing aldosterone and about half the cortisone is eluted from the chromatogram with methanol This eluate is rechromatographed in the toluene ethyl acetate aqueous methanol system (Bush 1951) to obtain the final separation of aldosterone

The detection and partial characterization of the steroid material

IN VITRO STUDIES OF THE FUNCTIONAL ZONATION OF THE ADRENAL CORTEX AND OF THE PRODUCTION OF ALDOSTERONE

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SINCE the discovery of aldosterone (Simpson *et al* 1953) the chemical and biological properties of this new hormone have been investi gated intensively However certain aspects of this problem such as the site of production of aldosterone within the adrenal gland the nature of the precursors of aldosterone and the mechanism of its secretion remain to be clarified

Direct proof that aldosterone is produced essentially by the zona glomerulosa of beef (Ayres *et al* 1956) and rat (Giroud Stachenko and Venning 1956) adrenals has been recently provided but such proof is still limited to these two animal species Wettstein Kahnt and Neher (1955) and Kahnt Neher and Wettstein (1956) have shown in adrenal homogenates that cortexone could be the precursor of aldosterone whereas in perfusion experiments Rosemberg and co workers (1956) have found that of cortexone corticosterone and progesterone only the last named produced a significant increase of aldosterone

Rauschkolb and Farrell (1956) have observed that the secretion of aldosterone in the adrenal venous blood of decapitated dogs is reduced when compared with normal controls The very recent demonstration by Farrell and co workers (1957) that beef hypothala mic extracts restore to normal the rate of aldosterone secretion of decapitated dogs strongly suggests that a humoral factor released by diencephalic centres might play an important role in the mechan ism of aldosterone secretion

The purpose of the present work is to study by means of *in vitro* methods two main problems of adrenal physiology The first is the functional zonation of the adrenal cortex i e the production of different types of corticosteroids by the different zones of the cortex

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with the connective tissue of the capsule The results of these experiments are condensed in Table I which compares the production of aldosterone of whole rat adrenals (0 66 ± 0 09 μg /100 mg tissue/hour) with that of the separated zona glomerulosa (1 8 ± 0 28 μg /100 mg tissue/hour) and of the fasciculata reticularis (0 14 ± 0 05 μg /100 mg tissue/hour) The last column of Table I gives the value of aldosterone production of the glomerulosa and of the fasciculata reticularis calculated on the basis of the total gland weight (0 53 ± 0 07 μg./100 mg. of tissue/hour)

Table I

COMPARISON OF *In Vitro* PRODUCTION OF ALDOSTERONE BY WHOLE RAT ADRENALS WITH THAT OF THE GLOMERULOSA AND THE FASCICULATA RETICULARIS

	Aldosterone (1) *μg /100 mg tissue/hour incubation*		
Whole glands	*Glomerulosa*	*Fasciculata-reticularis*	*Whole glands (calculated)* (2)
0 37	2 46	0 11	0 71
0 50	2 66	0 19	0 57
0 64	1 44	0 32	0 70
0 36	0 77	0 17	0 33
0 79	1 83	0 00	0 43
0 77	1 59	0 05	0 42
0 62			
1 20			
Mean 0 66 ± 0 09	1 79 ± 0 28	0 14 ± 0 05	0 53 ± 0 07

(1) Aldosterone was measured by the modified Singer Venning assay
(2) Calculated production of aldosterone on the basis of total tissue weight (glomerulosa + fasciculata reticularis)

From these findings it was concluded that aldosterone is produced by the zona glomerulosa of the rat adrenal cortex

The difficulty of obtaining the relatively large amounts of rat adrenal glands necessary to extend these observations persuaded us to pursue our investigation on beef adrenal cortex. The zona glomerulosa is separated from the two inner zones of these glands by cutting with a razor blade along a line of demarcation defined by a band of pigmented cells visible to the naked eye Serial histological sections of the outer zone show that it contains the whole glomerulosa and occasionally clusters of fasciculata The complete removal of

after chromatography is done by direct visualization and/or contact photography under u v light by the soda fluorescence (Bush 1952) the tetrazolium blue and the trimethyltetrazolium reactions on the chromatograms (Zaffaroni and Burton 1951) by mixed chromatogram with crystalline compounds and absorption spectrum in sulphuric acid solution (Zaffaroni 1950)

The quantitative determination of individual steroids bearing a Δ^4 3 ketone group is carried out in a Unicam spectrophotometer at 240 mμ (Saffran and Schally 1955) in methanol solution

Aldosterone is quantitatively measured by this method after the two chromatographic separations previously mentioned This procedure becomes possible without further purification in a third chromatographic system since the aldosterone fraction from the Bush C when reapplied on the *iso* octane *tert* butanol aqueous methanol system (Eberlein and Bongiovanni 1955) behaves as homogeneous Δ^4 3 ketonic material In each set of experiments aldosterone is furthermore characterized by its maximal absorption at 239 mμ in methanol solution by the single peak of its sulphuric acid chromogen at 288 mμ and by its biological activity in a modification of the Singer Venning assay (Venning Dyrenfurth and Giroud 1956)

The work concerning the action of posterior pituitary fractions on the secretion of cortisol and aldosterone *in vitro* utilizes the same methods except that whole beef cortical slices are incubated without separating the zona glomerulosa from the two inner cortical zones The different posterior pituitary fractions as well as ACTH are in all cases added to the media at the beginning of the incubation period

FUNCTIONAL ZONATION OF THE ADRENAL CORTEX

Our interest in the functional zonation of the adrenal cortex arose from the chance observation that when rat adrenal glands were incubated after decapsulation their secretion of aldosterone was insignificant (Giroud Stachenko and Venning 1956) This finding led to the assumption that decapsulation removes a part of the gland essential to the secretion of aldosterone That this is the case was proven when it was found that the capsules secreted aldosterone on incubation whereas the corresponding decapsulated glands did not and when it was established histologically that during the process of decapsulation the whole zona glomerulosa was removed

based on metabolic pathways specific to these zones Under these conditions identical precursors added to slices of beef adrenal cortex are handled in a radically different way depending on whether the slices originate from the zona glomerulosa or from the two inner cortical zones The results of three groups of experiments in which the precursor added was respectively cortexone progesterone and corticosterone at the dose level of 5 μg /100 mg of tissue incubated

Table III

PRODUCTION OF ALDOSTERONE, CORTISOL AND CORTICOSTERONE BY THE GLOMERULOSA AND FASCICULATA RETICULARIS OF BEEF ADRENAL INCUBATED WITH CORTEXONE PROGESTERONE AND CORTICOSTERONE (B)

Steroid added (1)	Glomerulosa				Fasciculata reticularis			
	Tissue weight incubated mg	μg /100 g tissue hr			Tissue weight incubated mg	μg /100 g tissue hr		
		Aldosterone	Cortisol	Corticosterone		Aldosterone	Cortisol	Corticosterone
Cortexone	2372	82	60 (2)	[illegible]90	3709	Nil	150	350
	1824	109	traces	576	3360	Nil	30	1[illegible]0
	1898	75	traces	130	[illegible]852	Nil	78	[illegible]80
	1892	127	traces	500	[illegible]882	Nil	70	340
Progesterone	[illegible]355	79	110 ( )	[illegible]50	3615	Nil	380	140
	1985	63	traces	75	3898	Nil	3[illegible]0	60
	1761	134	traces	298	3210	Nil	155	62
	1962	133	traces	490	3010	Nil	255	180
B	1795	115	traces	Not measured	3486	Nil	60	Not measured
	1674	2[illegible]0	traces	Not measured	2944	Nil	60	Not measured
	1976	142	traces	Not measured	3900	Nil	59	Not measured
Mean control production μg /100 g. tissue/hr		37 ± 5	traces	58 ± 8		Nil	61 ± 6	45 ± 9

(1) 5 μg /100 mg of tissue 2 hours of incubation.
(2) The detectable amounts of cortisol measured here were shown, by histological examination of the slices to be associated with less successful separation of the fasciculata reticularis from the glomerulosa than was the case in later experiments.
S E of mean

are shown in Table III In the lower part of this Table are found the mean values of cortisol aldosterone and corticosterone produced in control experiments in the absence of precursors

Cortexone progesterone and corticosterone increase the production of aldosterone by the glomerulosa but do not lead to the formation of this hormone in the fasciculata reticularis Only progesterone enhances the production of cortisol and this only in the fasciculata reticularis In both the glomerulosa and the fasciculata reticularis cortexone markedly increases the formation of

these fasciculata cells is made difficult by the fact that more careful dissection is limited by the time factor involved in the processing of a great number of slices However it is believed that this does not invalidate the conclusions from these experiments

In Table II are presented the results of a group of experiments which were undertaken to establish the basic production of cortisol aldosterone and corticosterone (expressed as µg/100 g of tissue/hour of incubation) by the zona glomerulosa and the fasciculata reticularis of beef adrenal From these data it can readily be seen that cortisol is produced almost entirely by the fasciculata reticularis

Table II

PRODUCTION OF CORTISOL ALDOSTERONE AND CORTICOSTERONE BY THE GLOMERULOSA AND THE FASCICULATA RETICULARIS OF BEEF ADRENAL

	Tissue weight incubated mg	*Cortisol µg/100 g tissue/hr*	*Aldosterone µg/100 g tissue/hr*	*Corticosterone µg/100 g tissue/hr*
Glomerulosa	7637	traces	48	38
Fasciculata reticularis	14101	68	nil	26
Glomerulosa	3920	traces	32	74
Fasciculata reticularis	7940	63	nil	40
Glomerulosa	4477	traces	44	69
Fasciculata reticularis	6890	47	nil	69
Glomerulosa	1919	traces	26	52
Fasciculata reticularis	2718	68	nil	45

although traces of this hormone for the most part too small to permit a spectrophotometric reading are found in the glomerulosa where they are most probably secreted by the clusters of fasciculata cells removed with the glomerulosa Aldosterone is found exclusively in the glomerulosa Corticosterone is secreted both by the glomerulosa and the two inner cortical zones

These observations confirm the original finding of Ayres and co workers (1956) on the site of production of these three steroids within the beef adrenal cortex

The results of experiments in which known corticosteroid precursors are used strongly suggest that the production of different types of hormones by the glomerulosa and fasciculata reticularis is

value of aldosterone produced in control experiments in the absence of precursor has already been recorded in Table III From this study it may be concluded that cortexone 11β hydroxyprogesterone and corticosterone increase the production of aldosterone that this is only observed in the presence of slices mainly if not exclusively composed of glomerulosa cells that the amount of aldosterone/100 g of tissue/hour of incubation is proportional to the amount of the steroid added at the doses tested (5 10 and 20 µg /100 mg of tissue) that the amount of aldosterone obtained by incubation with these

Table VI

PRODUCTION OF ALDOSTERONE BY THE ZONA GLOMERULOSA OF BEEF ADRENAL INCUBATED WITH CORTICOSTERONE (B)

Steroid added µg /100 mg tissue	*Tissue weight incubated mg*	*Aldosterone µg /100 g tissue/hr*	*Mean*
B 5 µg.	1795	115	
	1674	220	144
	1800	157	
	1800	172	
B 10 µg.	1722	274	
	1946	240	
	1868	200	250
	1868	240	
	2056	310	
	2056	240	
B 20 µg	1868	456	
	1932	400	414
	1932	380	

three steroids is similar and consequently seems to be unrelated to the pres nce or absence of an hydroxyl function at C 21 or C 11

These steroids do not lead to the formation of aldosterone when added to the incubation media of slices exclusively composed of fasciculata reticularis cells

When glomerulosa slices are incubated with 11 dehydrocorticosterone a compound with a chromatographic mobility very similar to that of aldosterone in the Bush B5 Bush C and E2B systems is obtained This unidentified steroid has been labelled compound III It has a maximum absorption at 239 mµ in methanol solution does not reduce tetrazolium salts and is devoid of biological

corticosterone whereas progesterone seems to enhance the production of this steroid mainly in the zona glomerulosa

Table IV

PRODUCTION OF ALDOSTERONE BY THE ZONA GLOMERULOSA OF BEEF ADRENAL INCUBATED WITH CORTEXONE

Steroid added µg /100 mg tissue	*Tissue weight incubated mg*	*Aldosterone µg /100 g tissue/hr*	*Mean*
Cortexone 5 µg	2372	82	
	2029	133	119
	1824	151	
	1824	109	
Cortexone 10 µg	1985	300	
	2274	214	
	2274	283	253
	2368	221	
	2368	290	
	2332	210	
Cortexone 20 µg	2269	370	430
	2269	490	

Table V

PRODUCTION OF ALDOSTERONE BY THE ZONA GLOMERULOSA OF BEEF ADRENAL INCUBATED WITH 11β HYDROXYPROGESTERONE

Steroid added µg /100 mg tissue	*Tissue weight incubated mg*	*Aldosterone µg /100 g tissue/hr*	*Mean*
11 OH progesterone 5 µg	1835	180	195
	1835	210	
11 OH progesterone 10 µg	1700	210	
	1615	293	
	1615	267	254
	1874	220	
	1460	280	
11 OH progesterone 20 µg	1700	318	

The specificity of the glomerulosa in forming aldosterone is further demonstrated in a series of experiments where this zone is incubated in the presence of cortexone 11β hydroxyprogesterone and cortico sterone The results are presented in Tables IV to VI The mean

The present investigation extends these previous studies and provides indirect proof that specific pathways of corticosteroid biosynthesis are to be found *within different layers of the adrenal cortex* This strongly suggests the existence of an enzymic specificity related to the elaboration of the steroid structures by the different layers of cortical cells Thus 17 hydroxylase activity would be essentially found in the inner zones of the cortex as suggested by the observation that progesterone increases the formation of cortisol in these zones but not in the glomerulosa the enzymic mechanism which leads to the formation of aldosterone would reside in the zona glomerulosa since corticosterone cortexone progesterone and 11β hydroxyprogesterone produce an increase of aldosterone when incubated with this zone but do not result in its formation in the presence of the fasciculata reticularis 11 and 21 hydroxylase activities would be distributed throughout the cortex as supported by the finding that in all three zones the formation of corticosterone is enhanced in presence of cortexone progesterone and 11β-hydroxy progesterone (the results with this last steroid have not been tabulated) Final proof of this concept will obviously have to await the results of experiments with enzymic systems obtained from the respective cortical zones

The problem of aldosterone precursors is still at the present moment a matter of controversy Wettstein Kahnt and Neher (1955) have shown that the yield of aldosterone by beef adrenal homogenates is increased in the presence of cortexone and decreased in the presence of corticosterone and progesterone Additional evidence for the role of cortexone as a possible precursor of aldosterone was obtained by Kahnt, Neher and Wettstein (1956) who demonstrated the conversion of [21 ^{14}C]cortexone to [21 ^{14}C]aldo sterone in beef adrenal homogenates In the last experiment the radioactivity formed by direct conversion accounted for 48 per cent of the theoretical total After addition of cortexone corticosterone and progesterone to the perfusion medium of isolated calf adrenal and subsequent biological measurement of aldosterone in the effluent Rosemberg and co workers (1956) concluded that of these three steroids only progesterone resulted in a significant increase of aldosterone cortexone did not qualify as a precursor of aldosterone corticosterone gave equivocal results The results obtained in the present studies differ from those of these two groups of workers in so far as they demonstrate a comparable increase of aldosterone

activity in both the Simpson Tait (1952) and the Singer Venning assays In fact in the last bioassay, compound III at the dose of 0 25 and 0 5 µg /rat promoted an increase in sodium excretion of 40 per cent above that of the control group In Table VII it can be seen that the amounts of compound III obtained in the presence of glomerulosa cells bear the same relationship to the three doses of 11 dehydrocorticosterone added as do the amounts of aldosterone to the three doses of 11β hydroxyprogesterone corticosterone and cortexone Further data are needed to establish the nature of compound III and to assess whether any significance can be attributed to its sodium excreting activity in normal or adrenalectomized animals Finally it

Table VII

PRODUCTION OF COMPOUND III BY THE ZONA GLOMERULOSA OF BEEF ADRENAL INCUBATED WITH 11 DEHYDROCORTICOSTERONE (A)

Steroid added µg /100 mg tissue	*Tissue weight incubated mg*	*Compound III µg /100 g tissue/hr*	*Mean*
A 5 µg	1703	150	
	1703	160	188
	1835	205	
	1835	240	
A 10 µg	1745	412	
	1745	412	396
	1411	418	
	1732	340	
A 20 µg	1475	440	440
	1475	440	

should be emphasized that incubation of the fasciculata reticularis with 11 dehydrocorticosterone does not result in the formation of compound III

Discussion

The present experiments provide new evidence to support the concept of a zonal specificity in the biosynthesis and secretion of different types of hormones by the glomerulosa and fasciculata reticularis of the adrenal cortex This concept which arose primarily from the classical studies of Deane Shaw and Greep (1948) received strong support when the secretion of specific hormones was demonstrated by incubation of different layers of the adrenal cortex (Ayres *et al* 1956 Giroud Stachenko and Venning 1956)

posterior pituitary (Infundin Burroughs and Wellcome which is *referred to as PPE in Tables VIII–X) and a posterior pituitary extract* prepared in Dr Saffran s laboratories It should be pointed out that the values of aldosterone obtained in these experiments are not directly comparable to those of the previous section since they are

Table VIII

ACTION OF POSTERIOR PITUITARY FRACTIONS ON THE PRODUCTION OF ALDOSTERONE AND CORTISOL BY BEEF ADRENAL SLICES

			Aldosterone	*Cortisol*
		Tissue weight incubated *g*	*µg /100 g /hr*	*µg /100 g /hr*
Control		11 46	10 0	60 0
PPE (1)	1 unit	11 3	25 0	90 0
PPE	4 units	11 5	30 0	100 0
Control		5 8	8 5	40 0
PPE	1 unit	7 0	20 4	60 0
PPE	4 units	6 5	30 8	75 0
Control		10 3	15 0	51 0
Post Pit Extract (2)		11 5	43 0	75 0
Control		7 5	11 5	80 0
PPE	1 unit	6 6	22 8	100 0
PPE	4 units	7 6	21 8	84 0
PPE	10 units	7 8	27 8	75 0
Control		8 5	6 0	40 0
PPE	1 0 unit	8 7	11 5	40 0
PPE	0 5 unit	8 6	15 6	50 0
PPE	1 0 unit	8 7	17 0	60 0
PPE	2 5 units	8 5	26 0	64 0

(1) PPE Posterior Pituitary Extract Infundin Burroughs Wellcome
(2) Available through the courtesy of Dr M Saffran.

expressed in terms of 100 g of whole cortex whereas the previous values are expressed in terms of 100 g of glomerulosa In a preliminary study 1 and 4 units of PPE are observed to enhance the production of aldosterone from a control value of 10 µg to 25 and 30 µg /100 g of tissue/hour of incubation whereas the production of cortisol is only slightly increased The results of two subsequent experiments show that the production of aldosterone increases from

production by the zona glomerulosa incubated in the presence of cortexone, corticosterone and 11β hydroxyprogesterone In the case of progesterone our data on the production of aldosterone are at the present moment too incomplete to be compared with those of the workers mentioned above

From the data presented in Tables IV–VI it can be calculated that the conversion of cortexone corticosterone and 11β hydroxy progesterone to aldosterone is in the range of 4 to 7 5 per cent irrespective of the three dose levels One is therefore tempted to conclude that in the sequence of reactions leading to the formation of aldosterone the oxygenation at the 18 position might be critical Such an hypothesis would have to be put to the test by the use of steroids bearing an hydroxyl group on C 18

PITUITARY FACTORS AFFECTING THE PRODUCTION OF ALDOSTERONE BY BEEF ADRENAL SLICES

Previous studies (Giroud *et al* 1956) have shown that the *in vitro* production of aldosterone by rat adrenals is slightly but significantly increased by the addition of ACTH to the incubation media whereas in the same conditions purified growth hormone has no action Recent experiments of Rauschkolb and Farrell (1956) and Farrell and co workers (1957) emphasized the role of the hypothalamus in the control of aldosterone secretion It was therefore thought of interest to investigate the action of different anterior and posterior pituitary fractions and it was hoped eventually hypothalamic extracts on the production of this hormone by beef adrenal slices

In the course of this investigation the production of aldosterone by whole beef adrenal slices was observed to be slightly enhanced in the presence of some crude extracts of beef posterior pituitary (Nordic Biochemical) In no experiments did this increase amount to more than 60 per cent of the control A comparable increase of cortisol was observed In view of the large dose used (20 mg /g of tissue incubated) even a slight contamination of these posterior pituitary extracts by ACTH could have accounted for these findings In addition the poor solubility of these fractions in Krebs Ringer solution made essential the use of more purified and soluble posterior pituitary extracts

In Table VIII are presented the results obtained with two different posterior pituitary fractions a commercial preparation of beef

posterior pituitary (Infundin Burroughs and Wellcome which is referred to as PPE in Tables VIII–X) and a posterior pituitary extract prepared in Dr Saffran s laboratories It should be pointed out that the values of aldosterone obtained in these experiments are not directly comparable to those of the previous section since they are

Table VIII

ACTION OF POSTERIOR PITUITARY FRACTIONS ON THE PRODUCTION OF ALDOSTERONE AND CORTISOL BY BEEF ADRENAL SLICES

		Tissue weight incubated g	Aldosterone µg /100 g /hr	Cortisol µg /100 g /hr
Control		11 46	10 0	60 0
PPE (1)	1 unit	11 3	25 0	90 0
PPE	4 units	11 5	30 0	100 0
Control		5 8	8 5	40 0
PPE	1 unit	7 0	20 4	60 0
PPE	4 units	6 5	30 8	75 0
Control		10 3	15 0	51 0
Post Pit. Extract (2)		11 5	43 0	75 0
Control		7 5	11 5	80 0
PPE	1 unit	6 6	22 8	100 0
PPE	4 units	7 6	21 8	84 0
PPE	10 units	7 8	27 8	75 0
Control		8 5	6 0	40 0
PPE	1 0 unit	8 7	11 5	40 0
PPE	0 5 unit	8 6	15 6	50 0
PPE	1 0 unit	8 7	17 0	60 0
PPE	2 5 units	8 5	26 0	64 0

(1) PPE Posterior P tuitary Extract Infundin Burroughs Wellcome
(2) Available through the courtesy of Dr M Saffran

expressed in terms of 100 g of whole cortex whereas the previous values are expressed in terms of 100 g of glomerulosa In a pre liminary study 1 and 4 units of PPE are observed to enhance the production of aldosterone from a control value of 10 µg to 25 and 30 µg /100 g of tissue/hour of incubation whereas the production of cortisol is only slightly increased The results of two subsequent experiments show that the production of aldosterone increases from

a control value of 8 5 and 11 5 µg to 30 0 and 27 8 µg. under the influence of 4 and 10 units of PPE Compared to the magnitude of aldosterone increase that of cortisol remains moderate Comparable results are obtained with Dr Saffran s posterior pituitary extract at the dose level of about 500 µg /g of tissue incubated An attempt is made to establish a dose reponse relationship between the production of aldosterone and increasing doses of PPE A reasonably good correlation is obtained in a single experiment with doses of PPE ranging from 0 1 to 2 5 units (See last experiment of Table VIII and Fig 1 for the chromatogram of the aldosterone fractions which correspond to this experiment.)

Precise data on the nature of Infundin and on its possible contamination with other pituitary hormones are not available at the present time However in addition to its main hormonal components, which are vasopressin and oxytocin it is highly probable that the preparation is contaminated by other pituitary hormones such as the melanocyte expanding hormone and judging from its action on cortisol detectable amounts of ACTH

To rule out the possibility that the observed increase of aldosterone produced by Infundin was due to ACTH pitressin or oxytocin commercial preparations of these hormones were tested *in vitro* under the same conditions Table IX shows that neither pitressin (Parke Davis) nor oxytocin (Parke Davis) has an influence on the production of aldosterone or cortisol In the second study of Table IX the production of aldosterone is not sensibly *affected in the presence of 0 1 and 0 2 International units of ACTH/* 100 mg of tissue incubated whereas the expected increase of cortisol occurs

It is of interest to note in the first study of Table IX that compared to the lack of action of oxytocin and pitressin the aldosterone-stimulating action of PPE is once more evident.

As previously pointed out by Wettstein Kahnt and Neher (1955) the yield of aldosterone by homogenates as well as by slices of beef adrenal markedly increases in the presence of cortexone Consequently it was decided to investigate if the production of aldosterone in the presence of this steroid and PPE would be greater than in the presence of cortexone alone To appraise the action of ACTH as a possible contaminant of PPE in the interpretation of the results ACTH was tested *concomitantly*

The results of these experiments are presented in Table X In



Fig 1 Photograph under u v l ght of a chromatogram showing the increasing amount of aldosterone obtained after add t on of 0 1 0 5 1 0 and 2 5 units of PPE (Infundin Burroughs and Wellcome) to the incubation media of beef adrenal slices starting from the control at the extreme r ght At the extreme left reference spots of cort sol and below cortisone (10 mg each) Chromatographic system toluene ethyl acetate aqueous methanol (Bush 195)

Table IX

ACTION OF PITRESSIN OXYTOCIN AND ACTH ON THE PRODUCTION OF ALDOSTERONE AND CORTISOL BY BEEF ADRENAL SLICES

	Tissue weight incubated g	Aldosterone µg /100 g /hr	Cortisol µg /100 g /hr
Control	7 7	24	45
Pitressin (1) 2 5 units	7 9	22	51
Pitressin 10 0 units	7 8	22	55
Oxytocin (2) 2 5 units	7 7	21	45
Oxytocin 10 0 units	7 7	21	48
PPE (3) 10 0 units	7 9	80	63
Control	7 9	18	44
ACTH (4) 0 1 I unit	8 0	23	90
ACTH 0 2 I unit	7 9	25	100
Oxytocin (2) 10 0 units	8 0	18	41
Oxytocin 20 0 units	7 9	22	47

(1) Pitressin Pitressin Parke-Davis Lot No 3-161-6
(2) Oxytocin Parke-Davis Lot No 3-160-6
(3) PPE Posterior Pituitary Extract Infundin Burroughs Wellcome
(4) ACTH Connaught Lot No 38-3 I U 0 1 and 0 2/100 mg tissue

Table X

COMPARISON OF THE ACTION OF ACTH AND POSTERIOR PITUITARY EXTRACT WITH AND WITHOUT ADDED CORTEXONE ON THE PRODUCTION OF ALDOSTERONE AND CORTISOL BY BEEF ADRENAL SLICES

	Tissue weight incubated	Aldosterone µg /100 g /hr	Cortisol µg /100 g /hr
Control	7 55	10	25
ACTH (1)	7 78	14	90
PPE (2) 10 units	7 8	33	
Control + cortexone (3)	7 94	34	42
ACTH + cortexone	8 1	43	85
PPE 10 units + cortexone	7 6	56	43
Control	7 9	22	31
ACTH	8 0	17	98
PPE 5 units	7 9	36	36
Control + cortexone	8 0	57	79
ACTH + cortexone	7 9	56	131
PPE 5 units + cortexone	7 9	82	

(1) ACTH Connaught 0 2 I U /100 mg of tissue
(2) PPE Infundin Burroughs Wellcome Lot No 4372
(3) Cortexone 10 µg./100 mg tissue/2 hours of incubation

brief they show that ACTH does not consistently increase the production of aldosterone by beef adrenal slices In both experiments, but to a lesser extent in the second PPE at the dose level of 10 and 5 units induces an increase of aldosterone from 10 and 22 to 33 and 36 µg respectively

In the presence of cortexone (10 µg/100 mg of tissue) the yield of aldosterone is enhanced threefold The addition of ACTH does not sensibly affect the production of aldosterone in the presence of cortexone whereas in the same conditions the stimulating action of PPE on aldosterone production is still demonstrable The production of aldosterone in the presence of cortexone is 34 and 57 µg/100 g of tissue/hour in presence of cortexone and PPE their values rise to 56 and 82 µg

Discussion

Studies on hypophysectomized animals have already indicated that the pituitary may play a role in the regulation of aldosterone secretion Less aldosterone was recovered from the adrenal venous blood of hypophysectomized rats (Singer and Stack Dunne 1955) and dogs (Farrell Rauschkolb and Koletsky 1955) than of intact controls Recently in a 13 year old boy with hypopituitarism the injection of human and monkey growth hormone promoted a marked increase of urinary aldosterone (Beck *et al* 1957) The possible participation of ACTH in the secretion of aldosterone is suggested from the results of a number of studies including *in vitro* work (Rosemberg *et al* 1956 Giroud *et al* 1956) as well as experiments in humans (Liddle *et al* 1955 Venning Dyrenfurth and Beck 1956)

The present study tends to show that certain commercial preparations of posterior pituitary contain a factor able to increase the production of aldosterone by beef adrenal slices It appears that this factor is different from the main known posterior pituitary components of these extracts since natural preparations of vasopressin and oxytocin are both devoid of activity in this system The observation that PPE (Infundin) increases the production of aldosterone two to threefold whereas that of cortisol is only slightly affected indicates that this factor is different from ACTH which has the opposite effect

Rauschkolb and Farrell (1956) have obtained indirect evidence that a centre localized in the diencephalon might play an important

part in the regulation of aldosterone secretion Farrell and co workers (1957) have lately substantiated this observation by showing that an extract of beef diencephalon given intravenously to decerebrated dogs increases the output of aldosterone in the adrenal vein blood

The data presented in this section are much too preliminary to lead to definite conclusions *Also the fact that only certain posterior* pituitary fractions and not all the batches of PPE (Infundin) have been found active in the conditions of the present study should remain our primary concern

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THE METABOLISM OF [16-³H]ALDOSTERONE IN MAN

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The possibility of making radioactive aldosterone of specific activity high enough for metabolic studies under physiological conditions arose from work on the mode and site of biosynthesis of aldosterone (Ayres *et al*, 1956 *a* and *b*) and the chemical synthesis of [16-³H]-progesterone of very high specific activity (Pearlman 1957)

PREPARATION OF [16 ³H]ALDOSTERONE

It has been shown that slices of whole ox adrenal cortex when incubated *in vitro* produce very small quantities of aldosterone compared with other steroids such as cortisol However relatively much greater amounts are made by incubating capsule strippings of the gland (Fig 1) These strippings consist of capsule plus zona glomerulosa tissue Similar work has been reported on the rat adrenal cortex (Giroud Stachenko and Venning 1956) Incubation of a single normal human adrenal has given results similar to those obtained with ox glands although the production of corticosterone was lower (Ayres *et al* 1957*c*) These preparations from ox gland also convert tracer amounts of [4-¹⁴C]corticosterone [21 ¹⁴C]cortexone and [4-¹⁴C]progesterone to radioactive aldosterone Larger quantities of [16-³H]progesterone (specific activity 1 7 μc per μg) are also converted to [16-³H]aldosterone in about 2 per cent yield (Table I) thus providing hormone sufficiently radioactive (about 0 9 μc per μg) for metabolic studies

The [16-³H]aldosterone was isolated following preliminary purification by chromatography on several high resolution partition columns Solvent systems similar to the Bush C and B5 (Bush 1952) were employed to purify the free compound which was then converted to its diacetate The derivative was chromatographed on another column and then hydrolysed the resulting free hormone

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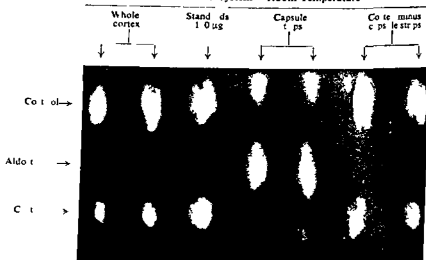


FIG 1 (See text)

DISAPPEARANCE OF [16 ³H]ALDOSTERONE FROM BLOOD

Two normal young males received intravenously 2 and 6 μg respectively of [16-³H]aldosterone at 9 a m Blood was collected from both subjects two hours later at 11 a m and from one subject also at 12 noon The radioactivity in the plasma was measured following (i) chloroform extraction (4 × 1 5 vol) at pH 6 (ii) chloroform extraction (4 × 1 5 vol) after incubation with β glucuronidase (1000 units ketodase per ml) at pH 4 5 and 47 C for 15 hours following precipitation of the proteins by alcohol (iii) continuous chloroform extraction at room temperature and pH 1 for 24 hours following precipitation of proteins This fractionation was similar to that employed by Migeon and co workers (1956 *b*) after injection of [4-¹⁴C]cortisol and corticosterone except that we used chloroform instead of ether for the third extraction at pH 1 Also all extracts were purified by silica gel chromatography as described by Bush and Sandberg (1953) before assay

The results for the free steroid fraction (i) in plasma obtained 2 hours after injection are shown in Fig 2 Corresponding values after [4-¹⁴C]cortisol and corticosterone obtained by Migeon and co-workers (1956*a* and *b*) and after [16 ³H]corticosterone by us are also shown After administration of [16 ³H]aldosterone the radioactivity in the free steroid fraction is much less than the corresponding results after administration of the other two radioactive steroids

The figures obtained by Peterson and Wyngaarden (1956) after administration of [4-¹⁴C]cortisol are even higher than those of Migeon and co workers (1956*a*) with the same steroid, although the former workers measured the radioactivity specifically as cortisol Migeon and co workers claim that at least one half of the radio activity in their free steroid fraction is due to cortisol After administration of [16 ³H]aldosterone about 80 per cent of the total radioactivity in the free steroid fraction in plasma 2 hours after injection was due to aldosterone Thus it seems that 2 hours after the injection of the two hormones radioactivity in the blood due to the administered steroid itself is about 20 to 30 times less after aldosterone than after cortisol The lower amount of radioactivity at this time may be due to a relatively greater miscible volume for the free steroid i e a greater fall in radioactivity during the initial mixing period (usually within 30 minutes of injection for cortisol) or three to five times shorter half life of the activity after equilibrium

being purified again on two columns The radiochemical purity of the final preparation was confirmed by bioassay and crystallization of a portion from added pure compound

SPECIFIC ACTIVITY OF ALDOSTERONE

The specific activity of the hormone or its derivative after column chromatography was estimated as follows Portions of the eluted fractions from the column were assayed for tritium by a planchet method in a windowless flow counter The amount of aldosterone or its diacetate in the fraction was estimated by measuring their fluorescence compared with that of standards by a fluorimeter following paper chromatography (Ayres *et al* 1957*b*) The specific

Table I

DATA ON STEROID PRODUCED BY INCUBATING 9 MG [16-^{3}H]PROGESTERONE (SPECIFIC ACTIVITY 1 74 μC PER μG) WITH 20 G CAPSULE STRIPPINGS OF OX ADRENAL GLAND VALUES CORRECTED APPROXIMATELY FOR RECOVERY

Steroid	*Amount in μg*	*Specific activity μc per μg*	*Percentage yield radio-activity*
Progesterone	2 800	1 39	39
Corticosterone acetate free	1 940	1 39 1 37	27
Aldosterone diacetate free	238	0 86 0 90	2 1
Cortisol acetate	35	0 58	0 2

activity in those fractions containing appreciable radioactivity could thus be measured and compared This served as another criterion for radiochemical purity as did a comparison of the specific activity of the diacetate and free compound which were found to be equal (Table I)

After administration of [16 ^{3}H]aldosterone the specific activity of the aldosterone extracted at pH 1 from urine was obtained by similar methods If a knowledge of the amount of radioactivity in an extract, present specifically as aldosterone was required then a known amount of inert aldosterone was added to the extract before processing and the value estimated from the measurement of the specific activity of the aldosterone after purification carried out as described above The methods have been reported in greater detail elsewhere (Ayres *et al* 1957*a*)

compared to 1 6-fold in the corresponding period for cortisol and 2 2 fold for corticosterone The half lives would therefore be 0 4, 1 4 and 1 0 hours respectively If the radioactivity is measured specifically as aldosterone the half life would appear to be even shorter about 0 3 hour These results indicate that more rapid metabolism or excretion of aldosterone compared with cortisol is the explanation

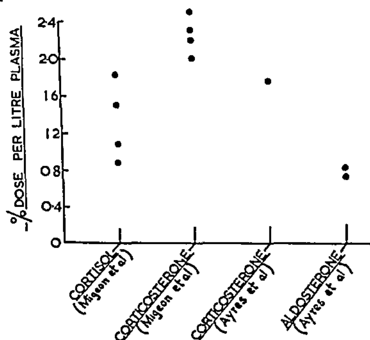


FIG 3 Radioactivity released by β-glucuronidase % dose per litre plasma 2 hours after injection of various radioactive steroids

Fig 3 shows the radioactivity released by β glucuronidase after extraction of the free steroids in plasma obtained 2 hours after giving labelled aldosterone with the corresponding values after radioactive cortisol and corticosterone administration The values obtained after injection of [16 ^{3}H]aldosterone are slightly lower than after administration of [4-^{14}C]cortisol and [4-^{14}]C- and [16 ^{3}H]cortico sterone Reincubation of the plasma with fresh enzyme gave only 10 per cent more radioactivity

Continuous chloroform extraction of blood at pH 1 and room temperature yields 0 15 per cent of the dose per litre plasma 2 hours

has been established This last explanation would mean that aldosterone is more rapidly metabolized or excreted compared with cortisol Ideally, the complete disappearance curves of the two hormones should be compared to decide this question but it was impossible to do this for aldosterone because of the low amounts of

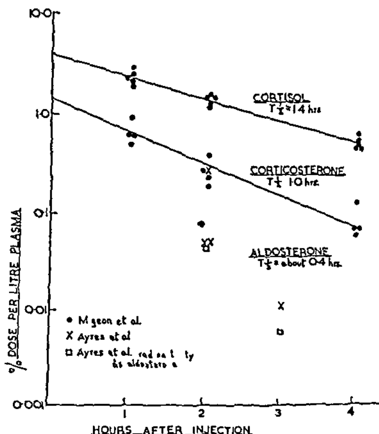


FIG 2. pH 6 $CHCl_3$-extracted radioactivity % dose per litre plasma measured 1 2 3 and 4 hours after injection of various radioactive steroids.

radioactivity in the blood Fig 2 also shows the radioactivity in the free steroid fraction of plasma for one subject, 2 and 3 hours after administration of [16-³H]aldosterone compared with values obtained at different times after administration of labelled cortisol and corticosterone The radioactivity is reduced sixfold during the period from two to three hours after the injection of labelled aldosterone

chloroform for 24 hours at room temperature using a Cohen type extractor It was then extracted by shaking with two 250-ml volumes of fresh chloroform which were pooled Both 500-ml extracts were washed with 100 ml 0 05 N Na_2CO_3 and 100 ml water which were both backwashed with 100 ml chloroform The combined chloroform extracts were taken to dryness *in vacuo* at 40 and further purified by passing through a 3 g silica gel column (Bush and Sandberg 1953)

All materials subsequently described as having been extracted at pH 1 were treated in this way Recovery of aldosterone added to urine was about 80% Dehydro*epi*androsterone was obtained in 25 % yield from a solution of its sulphate by this procedure

Extraction with chloroform at neutral pH Urine or other solutions were made up to 1 l and hand-extracted with one volume of 500 ml and two of 250 ml of chloroform The chloroform extract was then treated as for the pH 1 process

Extraction with ether alcohol The free and conjugated steroids were extracted from the urine by the method of Edwards Kellie and Wade (1953) Ammonium sulphate (50 g %) was added to urine acidified to pH 2 and the steroids extracted with a 3/1 (v/v) ether ethanol mixture (3 times with half volumes) The pooled ether ethanol extracts were evaporated to dryness at less than 40°C under reduced pressure The conjugate residue was dissolved in absolute ethanol filtered and transferred to the column after evaporation to 3 0 ml The ether alcohol extract can also be transferred to the column directly if the volume of urine is small enough

Experience with this method has shown that the 17 oxosteroids are extracted quantitatively and the corticoid oxogenic conjugates from 85–92 %

Adsorption column details

The column used is that which has been shown to separate quantitatively the free steroids the 17 oxosteroid glucuronides and the 17 oxosteroid sulphates from one another in unmodified form (Barlow 1957) The 17 oxogenic and 17 oxosteroid glucuronides appear in the same fraction

The column is prepared in benzene using neutral Woelm alumina (20 g height 20 cm internal diameter 1 2 cm) with a moisture content of 2 % estimated by weight lost overnight at 100 C After application of the extract in ethanol the column is washed with

after administration of [16 ^{3}H]aldosterone Whilst this value is higher than the corresponding one for [4-^{14}C]cortisol (0 05 per cent) obtained by Migeon and co workers by extraction with ether at pH I, it is much lower than those for the free steroid fraction after [4 ^{14}C]cortisol and the glucuronide fraction after [16 ^{3}H]aldosterone The amount of aldosterone released by this procedure was also very low and probably less than that released at neutral pH This is in contrast with the general experience with urine where much more aldosterone is extracted at pH 1 than at neutral pH

The radioactivity in the extract obtained by continuous extraction at pH 1 and by incubating with β glucuronidase was independent of the order in which the two procedures were carried out which argues against the existence in blood of large amounts of a mixed type of conjugate e g glucuronide at position 3 and sulphate at position 18 of aldosterone

It can be seen that the rapid metabolism of aldosterone has not been accounted for in terms of any radioactive metabolite in blood The quick disappearance from blood is unlikely to be due to a large miscible volume for the free steroid compared with that of cortisol However this may not apply to the metabolites of aldosterone whose miscible volumes may have a greater value than those of cortisol Attachment of a metabolite to a protein in the manner of the oestrogen ester complex of Szego and Roberts (1953) would tend to give such a value The protein could even be in blood for if the complex were to be of the same nature as that described by Szego and Roberts then the procedures used in the present work would not split it Another explanation of the rapid disappearance of aldosterone could be increased excretion of the free steroid or its metabolites We have no data on faecal excretion but if it were the explanation then it would be an unusual route for excretion of a steroid by man The data on urinary metabolites given below make it unlikely that increased urinary excretion of the free steroid or any glucuronide could be the explanation

RADIOACTIVITY IN URINE

Methods

Extraction with chloroform at pH 1 Urine without preservative (1 l) from a 24-hour collection or other material dissolved in 1 l of water was acidified to pH 1 and passed continuously through 500 ml

On reincubation, additional ketodase (350 Fishmann units/ml of urine equivalent) was added

When free radioactive aldosterone was treated by this procedure 76% was recovered as unchanged steroid

Saccharate inhibition

Saccharo-1 4-lactone was used as a specific inhibitor of β-glu curonidase (Levvy 1956) Potassium hydrogen saccharate in 0 1 M aqueous solution was boiled for 45 minutes and cooled just prior to use The solution so prepared was added to the 0 5 M acetate buffer containing the steroid conjugate so as to give a concentration of 0 05 M saccharate and the pH was readjusted to 4 8 Incubation after addition of enzyme phosphate and penicillin was then carried out as described above

Measurement of total oxosteroids

The method of Brooks and Norymberski (1953) as modified by Edwards and Kellie (1957) was used The 17α hydroxysteroids were first converted to 17 oxosteroids by the use of sodium bis muthate The final oxosteroids were removed from the aqueous medium by methylene dichloride The sulphates remain in the aqueous solution The method actually measures steroid glucu ronides which are initially present as 17 oxosteroids as well as those such as the corticoid glucuronides which are 17 oxogenic The figure obtained is one for total oxosteroid glucuronides

The urinary excretion of radioactive metabolites from one normal male was examined on two separate occasions On the first occasion he was on a normal salt diet and received 4 36 μg (3 7 μc) of [16 ³H]aldosterone intravenously Three months later he was depleted of sodium by sweating and placed on a low salt diet for five days until he was excreting only 7 m equiv sodium per day in his urine He was then injected with 2 35 μg (2 μc) of [16-³H]aldo-sterone intravenously Urine was collected from the time of injection for 24-hour periods and extracted and fractionated as previously described

Total radioactivity

Because of the difficulty of measuring tritium in the presence of large amounts of inert solids it was not possible to obtain the

50% benzene absolute ethanol (v/v 200 ml) This solvent usually brings off a large amount of non steroidal solid, some pigment and less than 2% of total oxosteroid (17 oxosteroids and 17 oxogenic steroids) This fraction usually contains the unconjugated steroids e g cortisone [16 ^{3}H]Aldosterone added to urine was recovered in this fraction in 92% yield It is subsequently termed the free steroid fraction

One hundred ml ethanol (first sulphate ' fraction) is then applied to the column which brings off more solid material but little total 17 oxo material The solvent is now changed again to 50% aqueous ethanol (v/v 150 ml) which elutes the 17 oxosteroid sulphates together with some pigment and the other solids Some 17 oxogenic steroids (1 3%) are also usually found in this fraction, which is subsequently termed the second sulphate fraction

Finally the 17 oxosteroid glucuronides and almost all (>70%) of the 17 oxogenic steroid glucuronides are eluted with 0 04 M Na HPO_4 citric acid aqueous buffer (pH 6 200 ml) The 17 oxosteroid and 17 oxogenic steroid conjugates are quantitatively recovered from the aqueous buffer by extraction with ether alcohol as described for urine Thus the bulk of the conjugates are recovered in this fraction (the glucuronide fraction) as a dry residue which weighs about 10% of the original extract and contains less inhibitory material for enzymic hydrolysis It should be noted that although these fractions are termed free glucuronide and sulphate and the 17 oxo sulphates and glucuronides and the 17 oxogenic glucuronides in urine behave according to this empirical classification the behaviour of 17 oxogenic sulphates or other types of hypothetical conjugates on the column is still unknown

Enzymic hydrolysis

The buffer eluted (glucuronide) fraction was incubated with beef liver glucuronidase (Warner Chilcott Ketodase 500 Fishmann units/ml of urine equivalent) Incubations were all carried out in 0 5 M sodium acetate acetic acid buffer (pH 4 8) at 37 C for the periods subsequently indicated and all incubations had the same total volume To each incubation mixture KH PO_4 is added to inhibit any sulphatase which may be present (Roy 1956) although according to Wotiz and co-workers (1957) steroid sulphatase is absent in this preparation Penicillin is also added to each flask After hydrolysis the released steroids are extracted with chloroform

Table III

RADIOACTIVITY OBTAINED BY DIRECT EXTRACTION OF URINE (2) AFTER INTRAVENOUS INJECTION OF [16-^{3}H]ALDOSTERONE TO SUBJECT (B W) ON LOW SALT DIET

2 35 µg. (2 02 µc) [16-^{3}H]aldosterone injected

First 24-hour urine specimen

	A	C	B
	pH 6 $CHCl_3$	pH 1 $CHCl_3$	Ether alcohol from $(NH_4)_2SO_4$ solution
5 4 % ^{3}H injection as 42 µg. aldosterone therefore secretion =780 µg. per day	Total 0 9 % ^{3}H	Total 11 % ^{3}H	Total 49 ± 2 % ^{3}H
	as aldosterone 0 16 % ^{3}H	as aldosterone 5 4 % ^{3}H (42 µg.)	82 % of total oxogenic and oxosteroids

All further values refer to the first 24-hour urinary collections the normal sodium diet urine is designated as urine (1) and the low sodium diet urine as urine (2)

Alumina column fractionation

About 93% of the radioactivity in the ether alcohol extract of urine (1) appeared in the buffer eluted fraction in which nearly all the 17 oxo and oxogenic glucuronides appear Less than 1 % of the injected radioactivity was in any other fraction (Table IV)

Table IV

RADIOACTIVITY OBTAINED BY FRACTIONATION OF ETHER ALCOHOL EXTRACT OF FIRST 24-HOUR URINE (1) (TABLE II) ON NEUTRAL ALUMINA COLUMN (BARLOW 1957)

B

Ether-alcohol extract from $(NH_4)_2SO_4$ solution of first 24-hour urine

Total 49 % ^{3}H — 85 % oxogenic

D	E	G	F
Benzene-alcohol free steroids	Buffer glucuronides	Aqueous ethanol sulphates	Ethanol sulphates
0 4 % ^{3}H	46 % ^{3}H	0 6 % ^{3}H	0 9 % ^{3}H
4 9 % oxogenic	72 % oxogenic	1 % oxogenic	1 4 % oxogenic

For urine (2) about 85 % of the ether alcohol radioactivity was in the glucuronide fraction and this time 5 % of the dose was in the second sulphate fraction (Table V) The nature of this metabolite was not further investigated

radioactivity of the crude urine. However, on ether alcohol extraction of the urine after the normal salt diet 49% of the injected radioactivity was recovered from the first 24 hour collection and another 10.4% from the next (Table II). Thus 59.4% of the radioactivity was accounted for in the first 48 hour urinary collection. Assuming recovery of these radioactive metabolites into the extract to be the same as for the corticoid oxogenic steroids, probably about 70% of

Table II

RADIOACTIVITY OBTAINED BY DIRECT EXTRACTION OF URINE (I) AFTER INTRAVENOUS INJECTION OF [16-^{3}H]ALDOSTERONE TO SUBJECT (B. W.) ON NORMAL SALT INTAKE

4.36 µg (3.7 µc) of [16-^{3}H]aldosterone injected

First 24-hour urine specimen		Second 24-hour urine specimen
A	B	
pH 6 $CHCl_3$	Ether alcohol extract from $(NH_4)_2SO_4$ solution	Ether alcohol extract from $(NH_4)_2SO_4$ solution
Total 0.4±0.2% ^{3}H; as aldosterone 0.4±0.1% ^{3}H	Total 49% ^{3}H injected; 85% urinary oxogenic material	Total 10.4% ^{3}H
C		
pH 1 $CHCl_3$	48 hour urinary excretion (extracted by ether alcohol) = 59.4%	
Total 8.5% ^{3}H	corrected approx. for recovery = 70%	
as aldosterone 3.8% ^{3}H (7.2 µg)	3.8% ^{3}H injection as 7.2 µg aldosterone therefore secretion rate = 190 µg per day	

the radioactivity was excreted in this form during this time. This is rather lower than the amount found in the crude urine after [4-^{14}C]cortisol by Migeon and co-workers. However it is not certain that all this ^{14}C radioactivity would be extracted by ether alcohol. On leaving the first 24-hour urine for twenty days at pH 1 and room temperature, a further 0.6% of the injected ^{3}H radioactivity was extracted.

After the low sodium diet 49% of the administered radioactivity was extracted by ether alcohol from the first 24 hour urine (Table III).

secretion rate of aldosterone is 190 µg per day on a normal salt diet and 780 µg. per day on a low salt diet.

Buffer eluted glucuronide ' fraction

The 3-oxo hormone conjugate from urine (1) gave 3 8% of the radioactive dose as aldosterone by extraction at pH 1 As no other fraction from the alumina column contained more than 1% of the dose it seemed likely that this conjugate was in the buffer eluate the glucuronide fraction Extraction of this fraction at pH 1 yielded

Table VI

RADIOACTIVITY OBTAINED BY EXTRACTION OF BUFFER FRACTION (TABLE IV) BY VARIOUS PROCEDURES, URINE (1)

E
Glucuronide fraction buffer eluted
Total 46% ^{3}H 72% oxogenic

H	I	J
pH 1 $CHCl_3$ 15 C	β-glucuronidase 64 hours at 37°C	pH 6 $CHCl_3$
Total 6% ^{3}H 5 1% oxogenic	Total 37% ^{3}H 76% oxogenic	Total 1 9% ^{3}H
as aldosterone 3 2% ^{3}H		as aldosterone < 0 2% ^{3}H

K (from I)
pH 1 $CHCl_3$ 15 C
Total 5 8% ^{3}H 5 1% oxogenic

^{3}H as aldosterone from pH 1 $CHCl_3$ extraction of urine=3 8%

3 2% of the dose as aldosterone (Table VI) compared with 3 8% as aldosterone after similar treatment of urine (Table II) Therefore presumably 84% of the 3 oxo hormone conjugate present in urine is recovered in the buffer eluted fraction Similar results on urine (2) indicate a recovery of 76% into this fraction (Tables III and VIII) Extraction of the buffer eluted fractions at pH 6 gave little radioactivity (< 0 2%) as aldosterone (Tables VII and VIII)

Thus it appears that this conjugate can be extracted by ether alcohol and is recovered from the buffer eluted fraction in about the same yield as the 17 oxogenic glucuronides It is also a definite complex which dissociates very little in the process

Chloroform extraction at neutral pH

0 4±0 2% of the dose for urine (1) 0 4±0 1% as aldosterone and 0 6±0 1% for urine (2) 0 15±0 1% as aldosterone were extracted by chloroform from urine at neutral pH The amount of radioactivity excreted as unchanged hormone is thus low in both cases as was the radioactivity in the free steroid fraction of the alumina columns (Tables IV and V)

Table V

RADIOACTIVITY OBTAINED BY FRACTIONATION OF ETHER ALCOHOL EXTRACT OF FIRST 24-HOUR URINE (2) (TABLE III) ON NEUTRAL ALUMINA COLUMN SUBJECT (B W) ON LOW SODIUM DIET

B
Ether-alcohol from $(NH_4)_2SO_4$ solution
Total 49% ³H 82% oxogenic

D	E	G	F
Benzene-alcohol free steroids	Buffer glucuronides	Aqueous ethanol second sulphate	Ethanol first sulphate
0 6% ³H 1 8% oxogenic	42% ³H 73% oxogenic	5 1% ³H 3 3% oxogenic	0/9% ³H 0 5% oxogenic

Radioactivity extracted by chloroform at pH 1

The amount of radioactivity extracted from urine (1) by chloro form at pH 1 following extraction at neutral pH was 8 5% of the dose included in this was 3 8% of the dose due to 7 2 μg aldosterone (Table II)

The radioactivity extracted from urine (2) at pH 1 was 11% of the dose of which 5 4% of the dose was due to 42 μg of aldosterone (Table III) No preliminary extraction at neutral pH was carried out in this case but a separate estimation showed that the free radio activity either total or as aldosterone (0 15%) would contribute little to the pH 1 values (Table III)

These results confirm the now general experience that much more aldosterone can be extracted at pH 1 than at pH 6 presumably because of hydrolysis of a conjugate henceforth termed the 3 oxo hormone conjugate Allowing for the recovery of aldosterone from this pH 1 extraction at least 4 5 and 6% of the daily secretion must be excreted in the form of this conjugate From the specific activity of the aldosterone released it can be calculated that the daily

aldosterone under these conditions of incubation but this may not have been due to enzymic action

To investigate this process further the glucuronide fraction from urine (1) was first extracted at pH 6 (Table VII Fig 4) Three

Table VIII

RADIOACTIVITY OBTAINED BY DIFFERENT TREATMENTS OF BUFFER FRACTION FROM SUBJECT (B W) ON LOW SODIUM DIET URINE (2)

E
"Glucuronide fraction buffer eluted
Total 42 % ^{3}H 73 % oxogenic

L(i)	M(i)	N(i)	H	J
acetate 0–24 hours	β-gluc. 0–24 hours	β-gluc. + saccharo-lactone 0–24 hours	pH 1 $CHCl_3$	pH 6 $CHCl_3$
Total 6 4 % ^{3}H 9 1 % oxogenic	Total 26 % ^{3}H 57 5 % oxogenic	Total 6 2 % ^{3}H 10 8 % oxogenic	Total 11 % ^{3}H	Total 6 6 % ^{3}H 18 % oxogenic
	as aldosterone 0 7 ± 0 2 % ^{3}H	as aldosterone 0 35 ± 0 1 % ^{3}H	as aldosterone 4 1 % ^{3}H	as aldosterone < 0 17 % ^{3}H
L(ii) acetate 24–48 hours	**M(ii)** β-gluc. 24–48 hours	**N(ii)** β-gluc. + saccharolactone 24–48 hours		**R** β-gluc. 72 hours
Total 0 6 % ^{3}H 1 4 % oxogenic	Total 4 8 % ^{3}H 11 4 % oxogenic	Total 0 8 % ^{3}H 2 2 % oxogenic		Total 19 % ^{3}H 52 5 % oxogenic
O β-gluc. 48–118 hours	**P** pH 1 $CHCl_3$	**Q** pH 1 $CHCl_3$		
Total 21 % ^{3}H 38 % oxogenic	Total 3 3 % ^{3}H 2 2 % oxogenic	Total 3 3 % ^{3}H 3 0 % oxogenic		
	as aldosterone 1 9 % ^{3}H			

equal aliquots were then incubated with acetate buffer acetate plus enzyme and acetate plus enzyme plus saccharo-1 4-lactone respectively They were incubated from 0–24 24–48 and 48–72 hours extraction being carried out at the end of these periods and additional enzyme added The radioactivity extracted by chloroform was 1 9% The radioactivity released with acetate buffer or acetate plus enzyme

From urine (1), 46% of the dose was present in the 'glucuronide' fraction and 37% of the dose was released by β glucuronidase incubation for 64 hours (Table VI) A further 6% of the dose was

Table VII

RADIOACTIVITY OBTAINED BY INCUBATING BUFFER FRACTION FOR DIFFERENT TIMES URINE (1)

E
Glucuronide fraction buffer eluted
Total 46% ^{3}H 72% oxogenic

J
pH 6 $CHCl_3$

Total 1 9% ^{3}H

as aldosterone < 0 2% ^{3}H

0–24 hours incubation at 37 C	L(i) acetate	M(i) β-gluc and acetate	N(i) β-gluc. and acetate saccharolactone
	Total 0 6±0 2% ^{3}H 5 5% oxogenic	Total 26% ^{3}H 64% oxogenic	Total 0 6±0 2% ^{3}H 21% oxogenic
24–48 hours incubation at 37 C	L(ii) acetate	M(ii) β gluc and acetate	N(ii) β-gluc. and acetate saccharolactone
	Total 0 5±0 2% ^{3}H 3 9 oxogenic	Total 2 6% ^{3}H 9 3% oxogenic	Total 0 4±0 2% ^{3}H 3 2% oxogenic
48–114 hours incubation at 37 C	L(iii) acetate	M(iii) β-gluc and acetate	N(iii) β gluc and acetate saccharolactone
	Total 0 4±0 2% ^{3}H 3 9% oxogenic	Total 1 5% ^{3}H 4 9% oxogenic	Total 0 4±0 2% ^{3}H 2 8% oxogenic

extracted at pH 1 following enzymic hydrolysis The release of the oxogenic steroids was quantitative and other evidence suggests that the radioactive glucuronides were also completely hydrolysed The data therefore suggest that most of the radioactivity can be hydrolysed by β glucuronidase but some can only be released by pH 1 extraction Detectable radioactivity (> 1% < 3%) was released as

enzyme and acetate plus enzyme plus saccharate for 0–24 and 24–48 hours (Table VIII Fig 5) Rather more radioactivity was released in the first 24 hours from the acetate buffer and acetate plus enzyme plus saccharate than for urine (1) This is presumably due to the preliminary chloroform extraction at pH 6 not having been carried out However just as for urine (1) complete inhibition of the

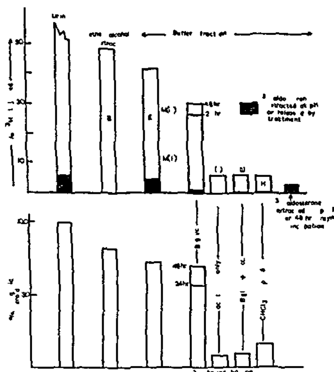


FIG 5 Fractionation of urine (2) (low sodium diet) for H and oxogenic steroids

enzymic hydrolysis was achieved by saccharolactone Only 0 7% of the dose as aldosterone was released by the enzyme incubation in the first 24 hours This represents only 20% of the radioactivity as aldosterone extracted at pH 1 Also in the first 24 hours 79% of the oxogenic steroids and 50% of the total radioactivity in the fraction were hydrolysed but not all this radioactivity was necessarily capable of being split by the enzyme Actually 80% of the total radioactivity released specifically by β glucuronidase in 48 hours was

plus saccharate was very small but detectable (<1%) in any period (Table VII) With enzyme plus buffer alone, 26% 2 6% and 1 5% of the dose were extracted in the three periods This indicates that the 64-hour hydrolysis of the radioactive glucuronides was complete 96% of the radioactivity released in the first 24 hours was inhibited by saccharolactone (Table VII) This is good evidence that the

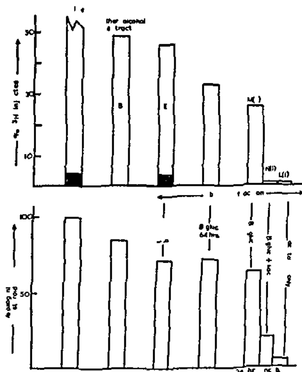


FIG 4 Fractionation of urine (1) (normal sodium diet) for H and oxogenic steroids

compounds responsible for this radioactivity were glucuronide metabolites The nature of these released free compounds [M(i) Table VII] will be discussed later Little radioactivity as aldosterone (<1%) was found amongst them This indicated that the aldosterone released in the 64-hour incubation might not be due to the action of β glucuronidase

The glucuronide fraction from urine (2) was divided into three equal aliquots and again incubated with acetate buffer acetate plus

(Fig 6) The positions of the peak concentrations of other standard steroids in the eluates of a similar column are also shown About one half of the tritiated compounds were very non polar probably *moving nearer the front than corticosterone, and these have not been* further studied They may be oxidative metabolic products of aldo sterone They are certainly conjugated with glucuronic acid as their release was inhibited by saccharolactone Most of the remaining radioactivity had exactly the same distribution in the eluted fractions

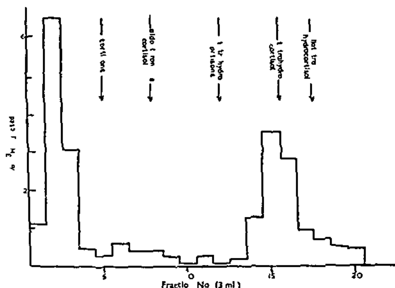


Fig 6 Steroids released by β-glucuronidase incubation for 24 hours of glucuronide fraction [M(i) Table VII]

as the tetrahydrocortisol in the extract although the presence of some radioactivity running at the same speed as *allo*tetrahydrocortisol is not excluded As aldosterone and cortisol run at exactly the same speed in this solvent system this radioactivity may be mainly due to tetrahydroaldosterone With complete enzymic hydrolysis about 12% of the injected radioactivity was present in this peak For the subject on the normal salt diet this would represent about 25 μg of steroid per day Little radioactivity was present in the more polar fractions A similar distribution of radioactivity on chromatography was found after enzymic hydrolysis of blood 2 hours after injection of [16-³H]aldosterone

hydrolysed in the first 24 hours pH 1 extraction of the acetate phase after the 48 hour incubation *with the ketodase yielded* 1 9% of the dose as aldosterone (Table VIII Fig 5) About one half of the conjugate, therefore was present even after this time of incubation Losses of the conjugate by extraction or destruction of the aldosterone by the processing must be considered In a control experiment [16 ^{3}H]aldosterone was incubated for 24 hours with enzyme under the same conditions and 76 % was recovered Also the possibilities of non enzymic hydrolysis or enzymic hydrolysis not due to β glucuronidase as indicated in the saccharate inhibited incubation must be taken into account It is therefore evident that little of the conjugate could have been hydrolysed by the specific *action of β glucuronidase under conditions* when quantitative *hydrolysis of the 17 oxo and oxogenic glucuronides* was achieved These results therefore indicate that either *the 3 oxo hormone* conjugate is not a glucuronide or it is one which is difficult to hydrolyse with β glucuronidase

Dehydro*epi*androsterone was 25% hydrolysed by these conditions of pH 1 extraction This steroid sulphate is one of the easiest to hydrolyse under a variety of pH 1 conditions It is difficult to give a comparable figure for the extent of hydrolysis of the 3 oxo hormone conjugate at pH 1 From urine (1) 46% of the dose was present in the glucuronide fraction which contained 85 / of the 3 oxo hormone conjugate 37% of the dose was released from this fraction by incubation with β glucuronidase for 64 hours At least 34% of the dose released by enzyme could be accounted for in steroids other than aldosterone Therefore a maximum of 12 % of the dose could be *the 3 oxo hormone conjugate As 3 2% of the dose* was released as aldosterone by pH 1 extraction this procedure would appear to hydrolyse 25% of the 3 oxo hormone conjugate as a minimum value Similar reasoning for urine (2) would lead to a higher figure

Thus the 3 oxo hormone conjugate would appear to be more difficult to hydrolyse with β glucuronidase than the known steroid glucuronides and more easily split by pH 1 extraction than the known steroid sulphates

The radioactive compounds released by β glucuronidase incubation for 24 hours of the glucuronide fraction [M(1) Table VII] were studied in preliminary work They were chromatographed on a partition column using the solvent system similar to the Bush C in which aldosterone and cortisol run at exactly the same speed

to be about 10 µg per day (Ayres *et al* 1957*b*) or about 5% of the daily secretion Estimation of the hormone itself has obvious technical advantages Enrichment of aldosterone compared with cortisol is obtained by acid extraction sensitive and specific methods for the estimation of 4 ene 3 oxones are available such as the Bush soda fluorescence reaction and bioassay procedures can be applied if necessary The only other definite metabolite found in urine is possibly tetrahydroaldosterone probably excreted at the rate of about 25 µg per day in normal man However this metabolite would be very difficult to separate from tetrahydrocortisol, which is excreted at about 1 mg per day The non polar glucuronide meta bolites might be unique products but they too must be excreted in small amounts

The 3 oxo hormone conjugate would therefore appear to be the most suitable metabolite to measure It has similar chromatographic properties on alumina to the 17 oxo and oxogenic glucuronides but not to the 17 oxo sulphates The chromatographic behaviour of 17 oxogenic sulphates is unknown The 3 oxo hormone conjugate of aldosterone is probably more easily split by pH 1 extraction with chloroform than the 17 oxo sulphates and less easily by β glucuro nidase than the 17 oxo and 17 oxogenic glucuronides A knowledge of the nature of the conjugate must await its isolation but its properties are not inconsistent with a sulphate at position 18 or 21 or even a conjugate of glucuronic acid which is not a β D gluco pyronuranide However it may be of an hitherto unsuspected type

There have been conflicting reports as to the relative efficiency of β glucuronidase incubation and pH 1 extraction in hydrolysing the conjugate The results reported here indicate that incubating the conjugate for 24 hours with a preparation of β glucuronidase which is relatively free of sulphatase and which has any residual sulphatase activity inhibited by phosphate is much less efficient than pH 1 extraction in releasing aldosterone This is so although under the same conditions the 17 oxogenic glucuronides including those of tetrahydrocortisone and tetrahydrocortisol are 80 / hydrolysed Appreciable hydrolysis however occurs when incubation is carried out for 64 hours These results indicate that the variable efficiency of incubation with β glucuronidase reported by different workers may be due to the use of an impure enzyme preparation or to release of aldosterone by a non-enzymic process In this case the amount of

About 34% and 27% of the injected ^{3}H radioactivity was present in urine (1) and (2) as glucuronide These values are slightly lower than those obtained by β glucuronidase incubation of the first 24-hour urine collection after administration of [4-^{14}C]cortisol (Migeon *et al*, 1956*a*) This indicates that increased urinary excretion of the glucuronide metabolites accompanying their increased synthesis is unlikely to be the explanation of the rapid disappearance of [16-^{3}H] aldosterone from blood

The results of these investigations would appear to be relevant to clinical studies as follows

ESTIMATION OF ALDOSTERONE OR A SUITABLE METABOLITE

Blood

The studies on the rate of disappearance of radioactive aldosterone and cortisol from blood indicate that the ratio of aldosterone to cortisol in peripheral blood is likely to be even lower (probably by a factor of three) than that in adrenal venous blood The secretion rates of the two hormones would appear to be about 0 2 mg. and 25 mg per day respectively The former value for aldosterone has been obtained for two normal young men by the methods described above Hence taking the mean daily plasma concentration of cortisol as 10 μg per 100 ml plasma that of aldosterone might be expected to be about 0 03 μg % This is in agreement with recent direct estimations (Ayres *et al* 1957*a*) using an isotope dilution method after addition of [16 ^{3}H]aldosterone to the blood *in vitro* The amount of hormone was not increased bv pH 1 extraction This presumably indicates a high renal clearance of the 3 oxo hormone conjugate compared with the free compound Thus unless larger amounts of the hormone can be extracted from the blood proteins its estimation in this fluid would not seem to be a practical clinical procedure

Although the glucuronide concentration is greater these metabolites would presumably lack the 4-ene 3 oxo structure and be present in minor amounts compared to the metabolites of cortisol

Urine

The results presented here confirm that as first found by Luetscher (1956) pH 1 extraction of urine splits a 3 oxo hormone conjugate and much more aldosterone (10–30-fold under our conditions of continuous extraction) is extracted at pH 1 then at pH 6 After acid extraction the normal mean excretion for 36 subjects has been found

Certain abnormalities in metabolism such as increased formation of both the 3 oxo hormone conjugate and the glucuronides might only be revealed by more detailed studies such as those used in the present investigation

Acknowledgements

This work was supported by the Medical Research Council Great Britain We thank Professor Sir Charles Dodds Professor J E Roberts and Dr J D N Nabarro for continuing their support and interest The work on the preparation of [16-^{3}H]aldosterone was carried out in collaboration with Dr W H Pearlman (Ayres Pearlman, Tait and Tait (1957) in preparation)

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hydrolysis would be expected to depend on a number of variables such as pH temperature and time of incubation as well as the nature of the enzyme used

INVESTIGATIONS OF RATE OF SECRETION AND MODE OF METABOLISM

The importance of a study of the metabolism of steroids as well as their rates of secretion has been emphasized by recent work of Samuels and Migeon and their co workers (Samuels *et al* 1957 Migeon *et al* 1957) Migeon s work indicates that the raised values of cortisol in blood in late pregnancy may be due to lowered metabolism rather than increased secretion Dobson (1957) claims that the rate of hepatic blood flow and hence rate of metabolism may be a factor in regulating aldosterone concentration in body fluids

The measurement of the 3 oxo hormone conjugate in urine has the disadvantage that at the most only 5% of the total secretion of aldosterone in the form of one particular conjugate is being measured and any abnormality of metabolism or renal clearance is not revealed By injecting [16 ^{3}H]aldosterone before the urine is collected a safe and simple procedure which could be carried out routinely the secretion rate of the hormone as well as the excretion of the conjugate may be measured The analytical method may also be simplified because the radioactive hormone is present throughout the procedure Preliminary studies indicate that the use of a high resolution column (approximately 1200 theoretical plates) using a Bush B5 solvent system, followed by paper chromatography of the peak fractions in the Bush C system provides a simple method which can be completed in three days

For semi routine purposes a comparison of the values for daily excretion of the aldosterone extracted at pH 1 (following preliminary extraction at pH 6) with the daily rate of secretion would reveal gross abnormalities of renal clearance or metabolism An example of this is provided in the present studies For a normal and low salt diet the daily excretions of the aldosterone extracted at pH 1 were 7 2 and 42 µg respectively and the daily secretion rates 190 and 780 µg respectively This shows that the rise in urinary aldosterone on sodium deprivation is a reflection of an increase in secretion rather than any altered metabolism or renal clearance It would be of great interest to extend these studies to such conditions as late pregnancy

throughout the whole beef adrenal cortex as previously demonstrated by Dr Tait s group and confirmed by our work Perfusion studies as well as incubation of slices have definitely shown that ACTH increases the production of this hormone Should we then expect that the production of corticosterone is under ACTH control in the fasciculata reticularis cells and independent of ACTH in the glomerulosa or could we possibly think that corticosterone is also under pituitary control in the zona glomerulosa whereas in this zone aldosterone is not?

I am tempted by the second hypothesis which if proven to fit with the facts would in my mind reconcile the general opinion according to which the pituitary does not control the secretion of the glomerulosa with the view of workers such as Lane and de Bodo and more recently Lever who demonstrated a partial regression of this zone after hypophysectomy

Wettstein Dr Giroud is your compound III which you get as a result of the incubation of 11 dehydrocorticosterone identical with the 11 dehydroaldosterone that Dr Genest claims to have isolated from urine of patients who were not given any precursors?

Giroud I do not know Dr Wettstein and I should like to prevent any confusion by saying that it is only by chance that the two compounds have been baptised with the same number At the present time our data on this compound are yet too incomplete to allow any conclusion concerning its chemical nature

Wettstein Your experiments concerning the action of posterior pituitary extract on production of aldosterone are very interesting Could you give us some information about Dr Venning s recent experiments with growth hormone?

Giroud You may remember that a few years ago Dr Venning showed that certain crude preparations of bovine growth hormone given intravenously to healthy subjects had a stimulating effect on aldosterone excretion When these experiments were repeated with Dr Li s highly purified preparations no effect on the excretion of aldosterone could be demonstrated Recently Dr Beck has studied two preparations of growth hormone a human growth hormone prepared from pituitaries obtained at autopsy and a monkey growth hormone both extracted by Dr Rabin (1957 *Science* **125** 884) These two fractions were administered intramuscularly to a 13 year old boy with a well recognized hypopituitarism. The patient was submitted to a complete metabolic balance study During the control period the urinary aldosterone was around 3 µg per day Over the 10-day period of human growth hormone injection his excretion increased progressively to reach a peak of 20 µg per day on the last day It then returned to the control value The same effect was noted with monkey growth hormone at which time the urinary excretion of aldosterone rose again reaching a peak of 14 µg per day at the end of a 6-day period of injection The patient received about 200 mg of human growth hormone and 350 mg of monkey growth hormone I believe that both Dr Beck and Dr Venning feel that their data do not disclose whether they are dealing with a specific effect of human and monkey growth hormone on aldosterone excretion or with some contaminant present in these preparations These results were confirmed recently in a second case of hypopituitarism

Gabrilove In connexion with Dr Giroud s most interesting paper I should like to bring up some points that are chiefly speculative in relation to the zones of production of the adrenocortical steroids Dr Giroud

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DISCUSSION

J F Tait I should like to compliment Dr Giroud on this beautiful work It has re-emphasized an important biosynthetic problem concerning aldosterone It arose first of all when Wettstein and his collaborators showed that cortexone was an intermediate in the biosynthesis of aldosterone If one then accepted the hypothesis of Hechter and Saba that ACTH acts on the breakdown of the cholesterol sidechain, then it was difficult to see why ACTH was relatively inefficient in increasing the secretion of aldosterone In 1956, we showed that tracer amounts of ^{14}C labelled progesterone cortexone and corticosterone were converted to radioactive aldosterone by capsule strippings of ox adrenal gland (Ayres *et al* (1957) *Biochem J* in press) One explanation of this migh have been that the enzyme system was already saturated and increased production of for instance corticosterone or cortexone by ACTH would not result in raised aldosterone secretion However we have recently found as has also been shown by Dr Giroud in his paper that excessive amounts of progesterone cortexone and corticosterone are very effective (and equally so) in increasing the production of aldosterone *in vitro* The next stage was the elegant demonstration in clinical studies at Geneva and Bethesda that ACTH does increase excretion for subjects on a low salt diet and that perhaps the lack of response to ACTH of aldosterone in normal man might be due to the electrolyte aldosterone controlling factor decreasing as a result of the increase in the so-called glucocorticoids by their effect on body electrolytes and spaces One could explain these results biosynthetically by the action of ACTH in increasing a precursor later than cholesterol such as cortexone or corticosterone and the conversion of cortexone or corticosterone to aldosterone being lowered by the suppression of the electrolyte factor However in this case ACTH should increase aldosterone production by the isolated gland and presumably also by incubated adrenal tissue without substrate ACTH should not increase aldosterone production when excessive amounts of a late precursor such as cortexone are present The crucial experiment therefore seems to be whether ACTH has an action without added substrate and Dr Giroud s results indicate that it has not. Therefore it would be interesting to know whether ACTH increases corticosterone production in the zona fasciculata but not in the zona glomerulosa and if the lack of stimulation by ACTH can be explained by such a preferential action

Giroud The work I have presented on the functional zonation of the beef adrenal has been done by Mrs Stachenko during the last eight months She studied first the basic hormonal production of the two separated parts of the glands then their production in presence of added precursors When I left Montreal recently she was only beginning to investigate the action of ACTH on the isolated glomerulosa and fasciculata reticularis respectively with and without the addition of corticosteroid precursors Now it is known that corticosterone is produced equally

not regenerate in the presence of a normal contralateral adrenal If you then remove the normal adrenal the enucleated gland will begin to regenerate and to form in about a month a fully normal cortex The presence of the pituitary is essential for this regeneration to take place The whole question has become even more interesting now that Dr Skelton has demonstrated that these adrenal enucleated rats develop a marked hypertension

Last year within the framework of our studies on the functional zonation of the adrenal cortex we began to investigate the corticosteroid secretion of the regenerating adrenal We used adult male rats removing the right adrenal and enucleating the left one The adrenal vein blood of the enucleated gland was collected 3 9 16 and 30 days after the operation It was found that a good correlation existed between the secretion of corticosterone in the adrenal vein blood of these animals and the histological appearance of the regenerating adrenal cortex It might be of interest to remark that as early as 3 days after operation a detectable amount of corticosterone (about one-tenth of the secretion of unilaterally adrenalectomized normal control rats) was present in the adrenal blood effluent of the enucleated adrenal and that the secretion of this hormone progressively increased over the period of observation to reach the value of the control after 30 days at which time the histological appearance of the cortex was that of a fully reorganized and regenerated gland without medulla of course

On the other hand and rather unexpectedly since the zona glomerulosa would seem to be the first zone to be fully regenerated the secretion of aldosterone was still definitely lower than normal after one month Soon we hope to be able to complete these experiments and to assess the meaning of these preliminary results

Gross We were very much intrigued by the enucleation hypertension produced by Skelton in his experiments Gaunt demonstrated that amphenone is unable to inhibit enucleation hypertension This would also favour the concept that aldosterone is not responsible for this type of hypertension We thought that probably corticosterone might be the responsible agent Therefore we tried to produce hypertension by over dosage of corticosterone in rats and we found that corticosterone can produce marked hypertension in rats

Giroud Dr Chappel did reduce the blood pressure of enucleated hypertensive rats by using amphenone (1957 *Endocrinology* 60 677) In fact he has shown that not only was amphenone able to make his animals normotensive but when started early enough after enucleation it prevented the anticipated rise of blood pressure Amphenone treatment under the same conditions had no effect on the blood pressure of adrenal ectomized animals made hypertensive by the administration of cortexone

Gross I am aware of these studies They are in contrast to the findings of Chart and Gaunt

Giroud It has been suggested that the differences between the results of Dr Gaunt and Dr Chappel might be explained on the basis of the dose of amphenone Dr Gaunt used 100 mg /kg whereas Dr Chappel used twice this dose

pointed out that in the zona glomerulosa there was aldosterone and corticosterone and in the zona fasciculata he reported cortisol and corticosterone Now there are several observations in experimental endocrinology which these findings might help to explain When ACTH is given to the experimental animal or to the human subject the adrenal cortex when examined pathologically resembles the zona fasciculata and the glomerulosa has almost disappeared When you administer cortisone or you hypophysectomize the animal or the human subject the zona fasciculata decreases markedly in size but the zona glomerulosa remains If you enucleate the adrenal cortex of a rat the adrenal cortex regenerates Although the regenerated cortex may appear anatomically somewhat different from the intact gland the regenerated gland functions normally in so far as the biogenesis of steroids is concerned according to Dr Dorfman In patients with hypopituitarism aldosterone production persists although it is often decreased In Farrell s hypophysectomized animals the output of aldosterone fell to about 66 per cent of normal If you give ACTH to an organism there is sometimes noted a slight increase in aldosterone elaboration concomitant with a marked increase in the urinary excretion of 17 hydroxycorticoids and the neutral 17 keto steroids

It has been claimed that the zona glomerulosa serves as a vegetative area for the cells that are elaborated later under the influence of ACTH to produce the glycogenic corticoids and the sexogens It would seem likely if we think about it teleologically that since the organism taken outside a marine environment must be primarily concerned with salt and water regulation this zone exerts a very primitive regulatory mechanism which must stay intact Later in evolution these vegetative cells perhaps under the influence of corticotropin differentiate into multipotent cells as hemic cells differentiate from stem cells which can elaborate new types of adrenocortical steroids exerting a facultative rather than an obligatory effect on mammalian metabolism These cells can then elaborate glucocorticoids and perhaps sexogens

It would seem likely that the zona glomerulosa is concerned with aldosterone secretion as its primary function but as Dr Giroud has shown corticosterone is also elaborated there as well as in the zona fasciculata Corticosterone would thus appear to be perhaps the stepping stone to the glucocorticoids In this regard it would seem that since adrenocortical tumours producing primary aldosteronism particularly carcinomas may elaborate hormones other than aldosterone and even corticosterone the purest type of aldosteronism may perhaps be sought in the patients with non tumorous primary aldosteronism

It has been claimed that regeneration of the adrenal cortex may arise either from the capsular blastema or the zona glomerulosa *depending on* the species I should like to ask since it is not clear from Dr Giroud s paper whether the hormonally functioning cells they employed are in the capsular blastema or in the zona glomerulosa ?

S A S Tait We can answer that question We have investigated capsule strippings with no adhering zona glomerulosa tissue and steroids were not produced

Giroud The growth promoting effect of ACTH on the adrenal cortex as related to the question of adrenal regeneration and corticoidogenesis is certainly a fascinating problem Quite a long time ago Dr Ingle showed that if you enucleate let us say the right adrenal of a rat this adrenal will

Contraction of the circulating volume was accomplished by performing phlebotomies of 700 ml once or of 500 ml twice Physiological saline equal in volume to that of the plasma removed was given immediately after each phlebotomy After periods of 12 hours to 6 days the red cells were reinfused the plasma having been removed In three studies salt poor albumin (25 g for each 350 ml of plasma removed) was added to the red cells in the remainder, the cells were injected undiluted One subject who demonstrated a complete inability to form serum albumin was subjected to plasmapheresis by phlebotomies 700 ml daily for 4 days with reinfusion of the red cells Albumin was later given to this subject to restore blood volume

Blood for *haematocrit haemoglobin total protein sodium* and potassium determinations was drawn at appropriate intervals The patient s arms were heated to arterialize the blood for haemoglobin and haematocrit determinations In some cases haemoglobin and haematocrit were determined at 4 hour intervals during control and experimental days

Haematocrits were determined in a microhaemocrit haemoglobins photometrically by the method of Crosby Munn and Furth (1956) For other methods employed see Bartter and associates (1956) Changes in blood volume were calculated in the albumin studies from the reciprocals of the haemoglobin concentrations they did not differ significantly from calculations based on the haematocrit values

RESULTS

A EXPANSION STUDIES

(1) *Albumin—short term*

Fig 1 shows the typical response of a normal subject to short term albumin therapy Aldosterone excretion fell and urinary sodium excretion increased The results were similar in the other two subjects studied in this manner

(2) *Albumin—long term normal subjects*

Fig 2 shows the response of a normal subject to long term albumin therapy Aldosterone excretion fell and the excretion of sodium rose Both effects were less marked in the later days of albumin infusion The response was similar in two other normal subjects the fourth showed equivocal changes in aldosterone excretion

EFFECT OF CHANGES IN INTRAVASCULAR VOLUME ON ALDOSTERONE SECRETION IN MAN

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U S Public Health Service Bethesda Maryland

ACUTE changes in body fluid volume can produce reciprocal changes in aldosterone secretion (Muller Riondel and Mach 1956 Bartter *et al* 1956) and the evidence supports the view that the concomitant changes in extracellular fluid volume are of primary importance in this type of regulation of the adrenal cortex

Changes in extracellular fluid volume *per se* could not however control aldosterone secretion unless the mechanism of control in patients with oedema and secondary aldosteronism were quite different from that in normal subjects as it clearly is not (Duncan Liddle and Bartter 1956)

Early studies had suggested that regulation depends ultimately on some function of intravascular volume (Bartter 1956) and the present studies were undertaken to explore this hypothesis

METHODS

All subjects were studied under metabolic regimen in air-conditioned environment of constant temperature Dietary sodium was low except in the plasmapheresis study *Expansion* of the circulating volume was accomplished by giving human salt poor albumin* (50 g a day) intravenously run in over 4 to 8 hours Two day courses were given to three normal subjects These three subjects received pitressin tannate in oil throughout the study to prevent the water diuresis which sometimes occurs when albumin is given and were kept recumbent Eight day courses have been completed in four normal subjects two patients with hypoproteinaemia (two studies each) and one with hepatic cirrhosis These subjects were ambulatory On the days albumin was given an amount of sodium equal to that in the albumin (13 m equiv /25 g of albumin) was withheld from the diet.

* We are indebted to the American Red Cross for the generous supplies of albumin for these studies

(3) *Albumin—long term hypoproteinaemic subjects*

Fig 3 shows the response of a woman with hypoproteinaemia to long term albumin therapy Aldosterone excretion fell sharply and excretion of sodium rose Both effects were less marked in the later

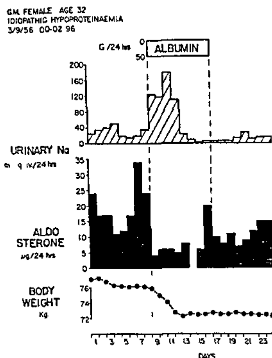


Fig 3 Effect of intravenous albumin on urinary sodium and aldosterone in a 32 year old woman with hypoproteinaemia

Bl d lume (not h w) had ris n to 109 pe ce t of contr l by day 11 and t 114 per ce t f co t l by d y 17 est mated fr m ha m toc ts

days of albumin infusion Although the results were thus qualitatively the same as those in the normal subjects quantitatively they far exceeded them Loss of sodium and of body weight indicated a marked increase in total extracellular fluid volume concomitant with the increase in intravascular volume The results were similar both qualitatively and quantitatively in the other study on this subject and in both the studies on the other hypoproteinaemic subject

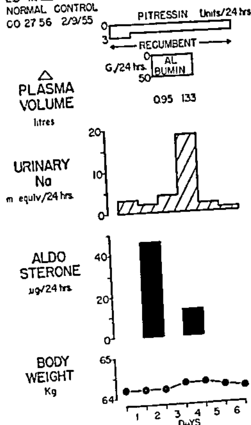


FIG 1 Effect of intravenous albumin on urinary sodium and aldosterone in a normal subject.

The estimates of plasma volume expansion assume an initial blood volume of 5 1 l

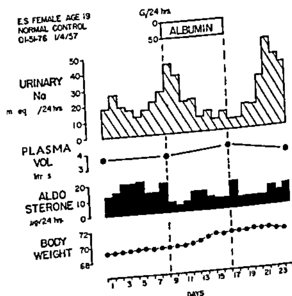


FIG 2. Effect of intravenous albumin on urinary sodium and aldosterone in a normal subject

The estimates of plasma volume assume an initial blood volume of 6 2 l

phlebotomy only the nocturnal output of aldosterone showed an increase after the second there was a further rise in aldosterone output after reinfusion aldosterone excretion fell and urinary sodium increased

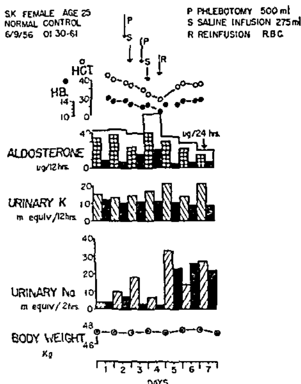


Fig 5 Effects of phlebotomy and red cell reinfusion on urinary aldosterone potassium and sodium and on circulating haemoglobin and venous haematocrit in a normal woman

Urine from 7 a.m. to 7 p.m. was analysed separately

Fig 6 shows the results of an experiment in a normal subject similar to the last except that three days elapsed between phlebotomies and four before reinfusion Aldosterone excretion rose with each phlebotomy but fell spontaneously thereafter after reinfusion of the red cells the lowest values of the entire study were observed

Fig 7 shows the results of an experiment similar to the last two in which phlebotomies were done on consecutive days and four days

(4) *Albumin—long term cirrhotic subject*

Fig 4 A shows the response of a man with hepatic cirrhosis to long term albumin therapy Aldosterone excretion fell sharply and sodium excretion rose Both effects continued throughout the period of injection Fig 4 B shows for comparison results from a previous study on this subject (see Duncan Liddle and Bartter, 1956) in which total extracellular fluid volume was expanded with physiological saline Aldosterone excretion was lowered to a comparable

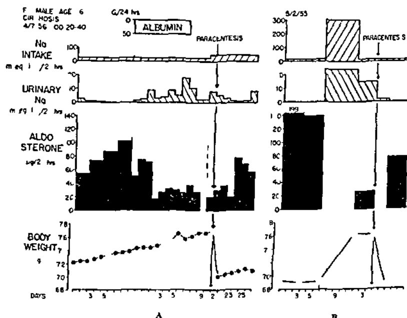


FIG 4 Effect of intravenous albumin (A) and physiological saline (B) on sodium and aldosterone excretion in a 46-year-old man with hepatic cirrhosis

Note that the paracentesis on day 21 did not lead to a significant rise in aldosterone secretion until time had elapsed for loss of fluid to the peritoneal cavity

B is from Duncan Liddle and Bartter (1956)

degree in the previous study but the total increment in body water was 7 litres on that occasion as opposed to 2 litres in the present study

B CONTRACTION STUDIES

(1) *Phlebotomy—normal subjects*

Fig 5 shows the responses of a normal subject to two phlebotomies and red-cell reinfusion done on consecutive days After the first

ten of the experiments reinfusion of red cells led to a further fall in aldosterone excretion

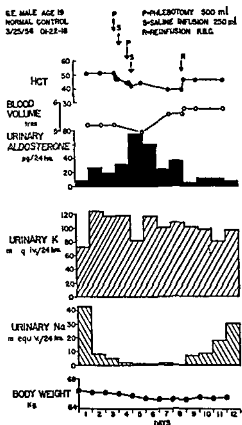


FIG 7 Effects of phlebotomy and red-cell re infusion on urinary aldosterone potassium, and sodium and on venous haematocrit in a normal man

The estimates f blood l me assume an initial volume of 5 2 l.

(2) *Plasmapheresis and albumin infusion—hypoproteinaemic subject*

Fig 8 shows the response to four daily phlebotomies followed by reinfusion of red cells in a hypoproteinaemic patient with a complete *inability to synthesize serum albumin* Aldosterone excretion rose progressively with plasmapheresis and fell with infusion of albumin

elapsed before reinfusion Aldosterone excretion rose with the second phlebotomy, fell spontaneously in the four day interval and fell still further with the reinfusion of red cells when appreciable quantities of sodium reappeared in the urine

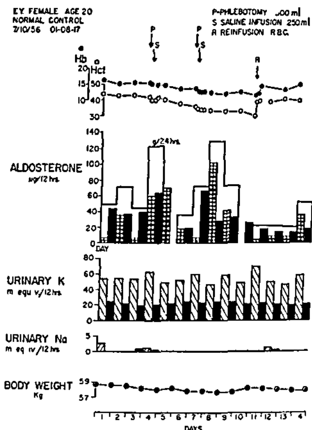


Fig 6 Effects of phlebotomy and red-cell reinfusion on urinary aldosterone potassium and sodium and on circulating haemoglobin and venous haematocrit in a normal woman

Urine from 7 .m. to 7 p m. was analysed separately

Twelve phlebotomy and reinfusion studies were done in all In ten aldosterone excretion rose following phlebotomy in two fluctuations in the control values obscured the results In the seven in which more than two days were allowed between treatments spontaneous declines of aldosterone excretion occurred In

DISCUSSION

These studies show decreases of aldosterone excretion with expansion and increases with contraction, of intravascular volume Glomerular filtration rate and renal plasma flow if affected at all should rise with the expansion and fall with the contraction Thus the changes in aldosterone excretion probably reflect blood levels, and not changes in renal function

In the normal subjects given albumin contraction of extracellular fluid (ECF) had been produced by moderate sodium restriction and aldosterone excretion in the control periods was higher than that found with high sodium diets Expansion of intravascular volume with albumin resulted in a lowering of aldosterone secretion to values within this normal range

The requirement that the control diets contain an amount of sodium equal to that later given with albumin made it impossible to restrict sodium rigorously and thus obtain very high control values for aldosterone secretion This accounts in part for the small changes in these subjects Re expansion of a contracted vascular volume to normal lowers aldosterone secretion but over expansion does not lower it further In the patients with hypoproteinaemia and cirrhosis control levels of aldosterone remained high despite moderate sodium intakes and the depressions with albumin were consequently very large The normal subjects differed markedly in the rate at which injected albumin left the circulation some showing little fall of *haemoglobin with the dosage used*

In the normal subjects who were phlebotomized sodium restriction was also used to elevate control aldosterone values Phlebotomy consistently produced a further rise in aldosterone excretion When more than two days were then allowed to elap e aldosterone excretion fell spontaneously this was invariably accompanied by haemodilution When the red cells were reinfused further falls in aldosterone excretion consistently occurred

The haemodilution which followed phlebotomy ranged from 17 to 35 per cent of the volume immediately after phlebotomy the time of maximum dilution ranging from 1 to 5 days These changes are similar to those observed by Wallace and Sharpey Schafer (1941) after removal of 700–1150 ml of blood from normal subjects Assuming an initial blood volume of 5000 ml these authors concluded that *maximum dilution corresponded closely to complete restoration* of the volume removed

C DIURNAL VARIATION

In seven studies day and night urine was analysed separately In four the excretion of aldosterone was higher during the day than during the night In one it was higher during the night than during the day and in two it showed no consistent pattern

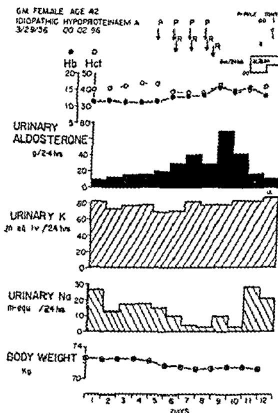


FIG 8 Effects of plasmapheresis and albumin infusion on urinary aldosterone potassium and sodium and on circulating haemoglobin and venous haematocrit in a 42 year old woman with hypoproteinaemia resulting from absence of albumin synthesis

Pla ma l mes as m ng an init al olum of 2 8 l we al w t d as 2 2 2 5 2 2 and 2 7 l on d ys 6 th o gh 9 of th s study

The f mula fo these calc lations is

$$PV = \frac{\left(\left[\frac{Hb \times PV}{1 - Ht}\right] - Hb \text{ r mo ed in g}\right)(1 - Ht_2)}{Hb}$$

where PV p esents plasma lum Hb h moglobin in g per 100 ml Ht haem tocrit in per ce t, and 1 and 2 two consecut days.

DIURNAL VARIATION OF ALDOSTERONE RELATED TO POSITION AND ACTIVITY IN NORMAL SUBJECTS AND PATIENTS WITH PITUITARY INSUFFICIENCY*

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FOURTEEN years have elapsed since the first demonstration by Pincus (1943) of a spontaneous diurnal rhythm in the urinary excretion of adrenal steroids Since then this observation has been well con firmed and carefully analysed in blood and urine by several groups of investigators This daily cycle appears fixed for any subject prone only to the variations superimposed by changes in the external environment These latter changes are mediated through the central nervous system and the anterior pituitary adrenocorticotrophic hormone So far neither the mechanism nor the significance of this diurnal rhythm of steroid levels is understood Since in pituitary insufficiency this diurnal variation is absent we assume that it is related to periodic discharges of the central nervous system through the anterior pituitary gland

In opposition to these often repeated observations concerning the diurnal rhythm of the corticoids there exist only limited data on the day and night cycle of aldosterone Until now the opinions differ

Venning Dyrenfurth and Giroud (1956) in a recent paper con clude that the majority of normal subjects have a diurnal variation of aldosterone similar to that of the 17 hydroxycorticoids However Luetscher and Curtis (1955) and Garrod Simpson and Tait (1956) do not confirm these findings We ourselves have been struck in previous studies by the regularity with which we have found a diurnal rhythm of aldosterone in most of our cases

In this paper we intend to analyse more carefully the problem of the spontaneous daily variation of aldosterone in healthy normal subjects and patients with pituitary insufficiency We shall also discuss briefly certain aspects concerning the homeostatic regulation of aldosterone in hypopituitarism

* This study was supported by a grant from the Fonds National suisse de la Recherche scientifique

When our data are calculated with this assumption complete restoration or overdilution appears to have occurred When however, they are calculated upon the assumption that blood volume is 6 per cent of body weight (Gunton and Paul 1955 Huff and Feller 1956) the same calculations show a final volume (before reinfusion) slightly below the initial one

Even after virtually complete spontaneous restoration of the initial volume red cell reinfusion lowered aldosterone excretion, because of sodium restriction the blood volume, when the red cells were injected, was still below normal for the subject on *ad libitum* sodium

SUMMARY AND CONCLUSIONS

Expansion of intravascular volume was induced in normal subjects and in patients with hypoproteinaemia and cirrhosis by the intravenous administration of salt poor albumin Contraction of intravascular volume was performed in salt depleted normal subjects and in one subject with hypoproteinaemia by means of phlebotomy red cells were restored to the normal subjects and albumin as well as to the subject with hypoproteinaemia

Expansion was followed by a decrease in aldosterone excretion in all subjects in whom it was initially elevated the contraction of intravascular volume was accompanied by increases in aldosterone excretion above the control levels

It is concluded that the regulation of aldosterone secretion by changes of body sodium and thus of extracellular fluid volume depends ultimately upon a function of intravascular volume

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[Discussion of this paper was postponed until after the paper by Muller and co-workers —EDS]

The experiment shows a definite diurnal rhythm of aldosterone identical with that of the 17 hydroxycorticoids Although a diurnal variation of potassium and water is observed throughout the entire study no regular pattern in the urinary sodium can be detected This lack of diurnal variation in sodium has been observed on several occasions when the subjects were ambulatory and on a relatively low sodium diet

It is conceivable that this constant diurnal variation of aldosterone *could be influenced by the sodium intake To settle this point* exactly the same experiment has been repeated in the same person but this time the 3 g of salt were given at night (second graph of Fig 1) It is evident that the two studies are identical in every respect To make certain however that the sodium intake has little if any effect on the diurnal rhythm of aldosterone this subject has consented to do a third experiment This time no metabolic control was attempted The subject had a varied and unrestricted diet containing from 2 to 8 g of salt per day Again the same diurnal rhythm was observed

In conclusion these experiments demonstrate a diurnal rhythm *in the urinary excretion of aldosterone a diurnal rhythm which is* identical with that of the 17 hydroxycorticoids Slight changes in the sodium intake do not alter this rhythm

The next step was to see how constant this rhythm was and how often it could be observed in normal subjects So far a total of 61 days and 61 nights has been examined in 4 normal subjects Higher excretions of aldosterone during the day than at night have been found on 53 occasions only on 8 occasions has there been absence of rhythm This clearcut result certainly depends to a great extent on the experimental conditions The determinations have not been done at random since in practically all instances the subjects have been on controlled diets and leading lives of regular activity

The third graph of Fig 1 shows one of these exceptional experiments with no diurnal variation despite a controlled diet Since on two previous occasions this subject had shown a very definite diurnal variation of aldosterone this result was rather surprising The only explanation one could think of for this lack of rhythm was the exceptional irregularity of the subject s life during this experiment He ate his meals at different hours his sleep was often interrupted and on two occasions he was up a good part of the night The authors confess that they were greatly influenced in this

1 DIURNAL VARIATION OF URINARY ALDOSTERONE IN NORMAL SUBJECTS

Fig 1 shows a metabolic study carried out in the following way The subject ate three meals each containing 1 g of NaCl at the same hours every day, he maintained his normal activity, working regular hours and always went to bed between 10 and 11 o clock at night The urines were collected in twelve hour periods from 7 a m to 7 p m, and from 7 p m to 7 a m

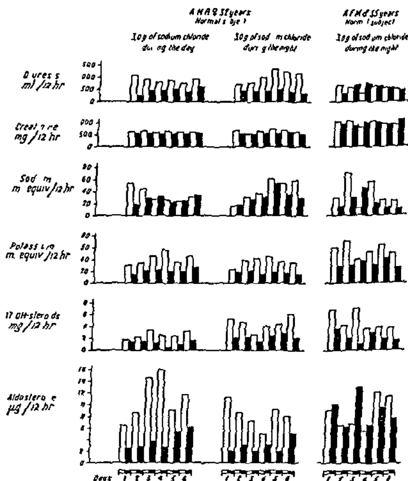


FIG 1 Influence of sodium intake on diurnal variation of aldosterone Note regular day and night pattern of aldosterone despite change in sodium intake as long as subject lives normal life Diurnal rhythm of 17 hydroxycorticoids and potassium constant throughout all experiments. No consistent diurnal rhythm of urinary sodium and no correlation with aldosterone

high urinary aldosterone values correspond to low urinary sodium, and *vice versa* This inverse ratio of the diurnal rhythm disappears with the upright position Activity and vertical position therefore,



FIG 2 Influence of position on diurnal variation of aldosterone The subject is always flat at night through out the entire study The comparison of position concerns only the day time collections Note discrepancy between day and night pattern of aldosterone and 17 hydroxycorticoids Excellent correlation between urinary aldosterone and sodium, *if subject is lying flat.* Note difference in sodium balance according to position

interpretation by an observation of Garrod, Simpson and Tait (1957 personal communication) These investigators had previously found an absence of diurnal rhythm in normal subjects who were recumbent throughout and for 36 hours before the experiment

To clarify these various points, a series of metabolic experiments during which the subject intentionally changed position and activity have been undertaken Fig 2 illustrates such an experiment Periods when the subject is up and active during the day and lying flat at night alternate with periods when he is inactive and lying flat day and night The changes in the diurnal rhythm of aldosterone are evident as a function of position and activity whereas the rhythm of the 17 hydroxycorticoids remains unchanged and does not depend on the same external factors However on the first day of bedrest only a very small and insignificant difference between the day and night excretion of the 17 hydroxycorticoids can be detected

This manifest discrepancy between aldosterone and corticoids points to a different origin of the two diurnal variations The rhythm of the 17 hydroxycorticoids seems to depend mainly on dis charges from the central nervous system whereas the day and night pattern of aldosterone with its variability according to position and activity probably does not reflect a basic physiological phenomenon It may be the mere consequence of our way of living i e being up during the day and in bed at night

In parallel but in inverse ratio with these changes in aldosterone excretion profound alterations in the urinary sodium were noticed The sodium balance is considered first equilibrated at the beginning the subject goes into negative balance the moment he lies down losing 49 m equiv of sodium in 3 days (low aldosterone) During the next 3 days the person is again ambulatory and he retains a total of 89 m equiv (high aldosterone) One day in bed reverses the situation 40 m equiv of sodium being lost When the subject resumes his activity the next day he again retains sodium In addition to these changes in the sodium balance considerable modifications in the day and night pattern of sodium have been observed A definite diurnal rhythm with a higher excretion during the day is found each time the person is inactive and in a horizontal position As soon as the subject resumes his activity this pattern disappears As to a possible correlation between the diurnal rhythm of aldosterone and that of urinary sodium one can detect one each time the subject is lying flat in bed Only under these conditions do

wonders if the increased production of corticosteroids during activity might play some role in establishing a rhythm To exclude this

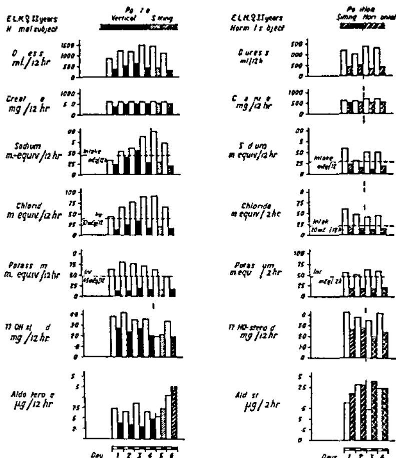


FIG 3 Influence of position and activity on diurnal variation of aldosterone The subject is always flat at night throughout the entire experiment. The comparison of position concerns only the day time collections Note in the first graph change in day and night pattern of aldosterone and 17 hydroxycorticoids while sitting (fifth day) Note decreased aldosterone excretion on the first day of lying flat in the second graph (third day) Regularity of water sodium chloride and potassium excretion is conditioned by diet (same meal every 6 hours throughout entire experiment)

direct effect of increased levels of corticosteroids cortisone has been given to a normal subject while in bed and no diurnal variation of aldosterone appeared An attempt to establish in two normal subjects a diurnal rhythm while lying flat by having them do

would seem largely responsible for the lack of correlation between the diurnal variation of the aldosterone and sodium excretions observed in this as well as in previous studies

Whereas this experiment irrefutably demonstrates the influence of position and activity on the urinary excretion of aldosterone and subsequently on sodium it does not allow a distinction between the relative importance of the two factors

In an endeavour to separate the influence of position from that of activity the following metabolic study was done (Fig 3) The same subject ate identical meals low in salt every 6 hours through out the experiment he always lay flat during the 12 hours at night but spent the days first up then sitting in a chair then sitting in bed with legs in horizontal position and finally lying flat From the moment that the subject becomes inactive and remains in a chair throughout the day the diurnal rhythm of aldosterone with its maximum excretion during the day disappears The 17 hydroxy corticoids maintain their diurnal rhythm except for a temporary disturbance on the first day of inactivity As regards the sodium excretion it shows a more distinct tendency to a diurnal variation on the days when the subject is inactive and sitting The day and night pattern of potassium chloride and water are not perceptibly influenced by the changes in position and activity

Considering this experiment it might be fair to say that the observed changes in the day and night pattern of urinary aldosterone excretion have essentially arisen as a result of change in activity and only to a minor degree as a result of change in position since from the haemodynamic standpoint the difference between standing and sitting with the feet on the floor seems small Nevertheless the in fluence of position cannot be completely excluded for in the second graph of Fig 3 we see that the aldosterone decreases temporarily during the period of a day when the subject changes abruptly from a sitting to a lying position The activity does not enter into consider ation here as the subject has already been inactive for several days

By what mechanism do position and activity influence the secre tion of aldosterone and produce a diurnal rhythm?

Every change in position alters the haemodynamic state in the body s vascular system resulting in changes of volume as well as of tonus of the vascular tree which could conceivably influence the secretion of aldosterone A change in activity could equally well have a repercussion on the vascular system But in addition one

Before discussion of this point the relative independence of the regulation of aldosterone from the pituitary will be illustrated in the next two figures

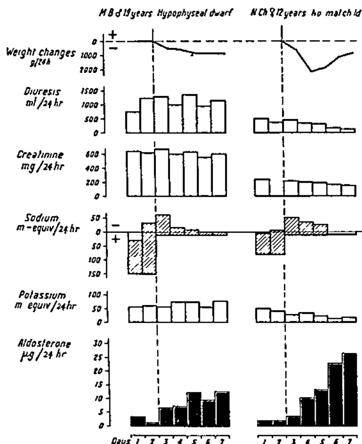


FIG 4 Comparison between salt restriction in hypophyseal dwarf and normal child Note *large fluctuations in weight, sodium and aldosterone in the child* as opposed to the dwarf

Fig 4 shows the response of a hypophyseal dwarf to sodium restriction The gradual increase of aldosterone is clearly visible but if compared with that in a normal child of the same bone age certain differences are immediately apparent The changes in weight as well as in sodium excretion are less abrupt in the dwarf however at the end of 5 days the weight change and the sodium loss are the

regular bicycling exercises was also unsuccessful It would seem therefore that both change in position as well as in activity affect ultimately the secretion of aldosterone in a similar manner i e through modifications and their consequences in the volume and the tonus of the vascular tree

Conclusions

These experiments demonstrate

(1) The diurnal variation in the urinary excretion of the 17 hydroxycorticoids and its relative independence of muscular activity and position have been confirmed

(2) A greater excretion of aldosterone during the day than at night has been observed in the majority of healthy subjects leading a normal life Slight changes in the sodium intake do not noticeably influence this diurnal rhythm

(3) Unlike that of the 17 hydroxycorticoids the diurnal variation of aldosterone is not constant since inactivity and horizontal position abolish it Therefore the two diurnal rhythms do not have the same aetiology and significance Whereas the former is a basic physiological phenomenon the latter seems to be the mere consequence and reflection of muscular activity and vertical position

(4) This interpretation explains the apparently contradictory statements in the literature by Venning Luetscher and Garrod

(5) This decrease in urinary aldosterone and increase in urinary sodium during the day when the subject was in bed gives a scientific basis for a long known clinical observation i e the beneficial effect of bedrest in all those diseases in which sodium is actively retained and oedema occurs On the other hand sodium retention and oedema will be much more severe in these patients if they are up and active

(6) The well known postural antisaluresis therefore can be mediated through the adrenal gland by way of its aldosterone secretion

2 DIURNAL VARIATION OF URINARY ALDOSTERONE IN PATIENTS WITH PITUITARY INSUFFICIENCY

Since the regulation of aldosterone is to a great extent independent of the pituitary it could be predicted that in pituitary insufficiency the daily pattern of aldosterone is maintained if the patient is up and active whereas the diurnal variation of the 17 hydroxycorticoids would tend to disappear

satisfactory sodium retention and increased excretion of aldosterone But, whereas the hypophyseal dwarf attained urinary aldosterone values between 10 and 13 μg /24 hours these two patients levels never exceeded 7 and 3 μg /24 hours Therefore the response to salt restriction in hypopituitarism i e the increased excretion of aldosterone varies according to the different grades and severity of pituitary insufficiency It is perfectly conceivable that there exist cases of hypopituitarism which do not adequately respond to salt restriction in increasing their aldosterone values only insignificantly or not at all

The second experiment resembles in many respects the case published by Wynn and Garrod (1955) When cortisone is stopped and salt is restricted a negative sodium balance appears in their case 320 m-equiv in this one 340 m-equiv However the accompanying loss of body weight is much less than expected Unfortunately the plasma sodium has not been determined Certainly the same severe hypotonicity would have been found as in Garrod s and Wynn s case Their patient s sodium level dropped as low as 107 m-equiv /l Before cortisone therapy was resumed increasing amounts of *endogenous* cortisone have been produced by 4 days of ACTH therapy The eosinophils dropped from 125 to less than 5/mm³ in 4 days There was a prompt water diuresis with a concomitant loss of weight of 1 kg 300 g Yet sodium was retained Needless to say the patient s clinical improvement was manifest The previous symptoms of weakness and lethargy disappeared It is interesting to see that the aldosterone not only increased during ACTH therapy but remained at high levels when ACTH was stopped and cortisone resumed One is entitled to consider this increase of aldosterone as a homeostatic response to the weight loss and not as a direct stimulation by ACTH This observation is also very significant in another respect it shows that a sodium loss *per se* unaccompanied by the corresponding weight loss causes a smaller increase in the secretion of aldosterone than is caused by the same sodium loss *with* the corresponding water loss This experiment which nature carries out can be reproduced artificially in a normal subject by preventing with pitressin the weight loss which always accompanies salt restriction Fig 6 illustrates such an experiment In the first graph the normal sequence of events to sodium restriction is shown *momentary sodium loss—weight loss—increased aldo*sterone excretion In the second experiment to the right pitressin

same i e both lose 800 g and between 80 and 90 m equiv of sodium Yet the hypophyseal dwarf accomplishes this homeostatic regulation with considerably less aldosterone than the normal child It seems therefore that the patient with pituitary insufficiency has a more sensitive and probably also a more delicate regulatory system than

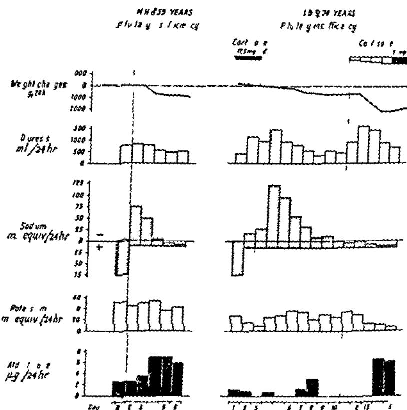


FIG 5 Salt restriction in severe pituitary insufficiency Note relatively small weight change which accompanies a marked sodium loss Cortisone corrects salt and water discrepancy and at the same time increases aldosterone Note pure water diuresis with ACTH (endogenous cortisone)

the normal child who better tolerates these large fluctuations in weight and sodium

Fig 5 illustrates the response to salt restriction in two patients with severe pituitary insufficiency both are unable to live for a prolonged period without replacement therapy whereas the dwarf has never needed hormonal therapy The metabolic pattern is essentially the same as before minimal weight loss active and

hyponatraemia and relative water intoxication Cortisone in its permissive role corrects these metabolic disturbances Therefore not only an intact and functioning aldosterone mechanism but also the presence of cortisone in its permissive rôle are prerequisite to a normal response to sodium restriction

Following this brief digression on the physiopathology of hypopituitarism the diurnal variation of aldosterone will be considered in this situation On the basis of the preceding observations on normal persons the same diurnal variation was expected in pituitary insufficiency as in healthy persons if the patients were leading a life *of normal activity*

Fig 7 illustrates two metabolic experiments one in a hypophyseal dwarf and the other in a 58 year old man with severe pituitary insufficiency The diurnal rhythm of aldosterone is absent in both patients Whereas the hypophyseal dwarf still has a day and night pattern of the 17 hydroxycorticoids the other subject has neither a diurnal rhythm of aldosterone nor of the 17 hydroxycorticoids Despite this lack of rhythm in aldosterone the urinary sodium excretions show a very marked rising and falling rhythm This observation strengthens the concept that the diurnal variation of urinary sodium depends only to a certain extent on the aldosterone and *that other probably non hormonal factors also intervene* The well known night diuresis of these patients is also clearly visible The potassium excretion however shows the pattern of a normal person

Now the question is why do patients with pituitary insufficiency not have a diurnal variation of aldosterone? And what will give them one? Is it because they lack ACTH? Or is it because they lack cortisone?

If ACTH is given and a rhythm appears one would never know whether this rhythm is due to the ACTH itself or to the endogenous cortisone produced by the ACTH Therefore cortisone has been given Fig 8 shows two such experiments in a hypophyseal dwarf The results are self evident Cortisone (in this case it was prednisone) established the diurnal variation of aldosterone on every occasion

By what mechanism does cortisone produce a diurnal rhythm of aldosterone in the hypophyseal dwarf when he is up and active? Could it by any chance be that the organism transforms cortisone into aldosterone? Or could it be that cortisone influences directly the secretion of aldosterone? Or thirdly is it only through the presence

prevents the weight loss but nevertheless sodium is lost in excess even more than in the preceding study yet the urinary aldosterone excretion does not increase

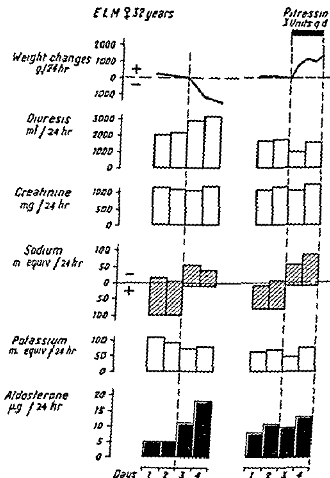


FIG 6 Abnormal response to salt restriction in a normal subject because of pitressin administration which prevents weight loss

In conclusion the patient with pituitary insufficiency seems perfectly capable of adapting himself to a decreased salt intake because of the ACTH independent homeostatic regulation of aldosterone However in the course of this adaptation discrepancies between the sodium and water metabolism may occur with resulting

Then the subject goes to bed for 2 days and despite prednisone no diurnal rhythm appears The last two days the patient is up and active, but since he is still taking prednisone a diurnal rhythm now appears The second graph of Fig 8 shows a similar study in a

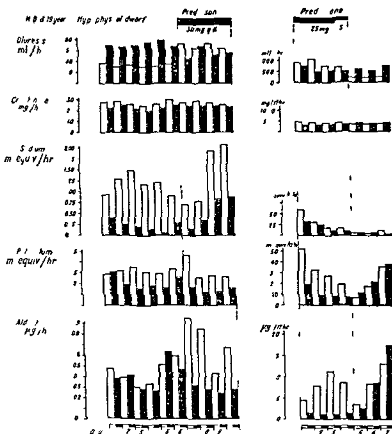


FIG 8 Influence of pred sone on d urnal variation of aldosterone Note appearance of rhythm without marked modifications in sodium and potassium excretion particularly in the first graph Note change in diurnal pattern of diuresis while on prednisone

normal subject The person is in bed during the entire experiment As expected the diurnal variation of aldosterone is lacking throughout despite two days of prednisone therapy which produces essentially no change in the urinary excretion of aldosterone These experiments exclude a direct metabolic action of cortisone on the

of cortisone that the patient with pituitary insufficiency establishes a diurnal rhythm, when he is up and active?

Offhand the first two possibilities are less likely but only an experiment based on the following reasoning can exclude them i e

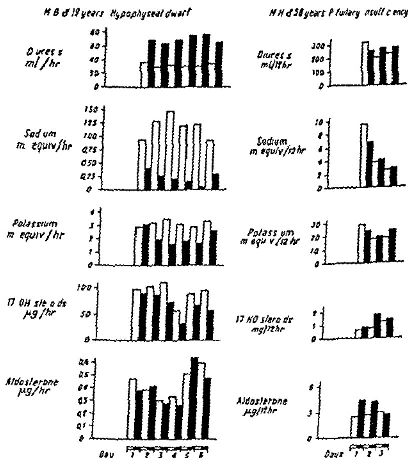


FIG 7 Diurnal variation of aldosterone in pituitary insufficiency Note absence of rhythm of aldosterone in both subjects as well as of 17 hydroxycorticoids in second patient. Day and night pattern of sodium is particularly marked in hypophyseal dwarf note also night diuresis in the same patient

that cortisone will not produce a diurnal rhythm of aldosterone in a person with pituitary insufficiency when he is in bed although it does so when he is up and active Fig 9 illustrates this final experiment For the first 3 days the patient is leading a normal life without cortisone as expected no diurnal variation of aldosterone is present

Conclusions

These experiments demonstrate

(1) The patient with pituitary insufficiency is capable of adjusting his urinary sodium excretion to a decreased salt intake because of his intact homeostatic regulation of aldosterone but cortisone in its permissive role is prerequisite to a normal response Otherwise discrepancies between the sodium and water metabolism may occur in the course of this adaptation

(2) The patient with pituitary insufficiency does not show a diurnal variation of aldosterone with a higher excretion during the day even when he is ambulatory and active

(3) Cortisone establishes the diurnal rhythm if the patient is up and active but not if the subject is lying flat This is an example of what Ingle called the permissive action of cortisone

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DISCUSSION

Garrod Dr and Mrs Tait Dr G Walker and I have studied two physiological situations in which very low levels of aldosterone secretion might be expected taking advantage of the sensitivity of the method we are using These situations are diurnal variations and salt loading Fig 1 shows the absence of any significant diurnal variation in aldosterone secretion in three normal subjects who were recumbent throughout the tests and who had been allowed 36 hours in which to equilibrate in a recumbent position before the 8 hour urine collections were started They were on a 4-hourly diet of constant composition and of normal sodium and potassium content The shaded areas represent the night periods from 10 p m. to 6 a m. and the unshaded periods the day time It will be seen that they show the usual anticipated large diurnal variations in sodium and potassium excretion low during the night high during the day but there is really no significant pattern of aldosterone excretion between the day and the night or between one 8 hour period and another whereas there is this very marked fall of cortisol excretion during the night with a large rise during the early period of the day

Fig. 2 shows the effects of giving 20 g of NaCl per day for two days to two normal subjects who were ambulant whilst carrying out their normal duties and on a normal diet. The 2 day control periods show the cortisol aldosterone potassium and sodium excretions and the body weight before

secretıon of aldosterone as well as a chemıcal transformatıon of one hormone to the other, sınce cortısone alone does not produce a rhythm Therefore, ıt ıs the vertıcal posıtıon and the muscular

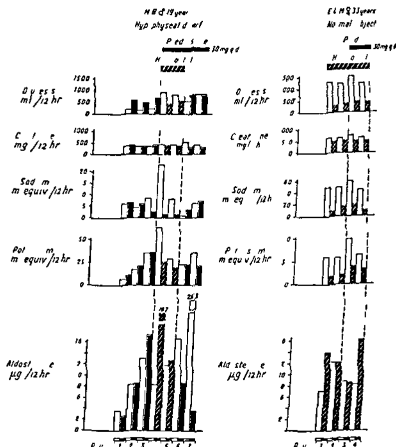


Fig 9 Influence of prednısone on dıurnal varıatıon of aldosterone ın a hypophyseal dwarf and a normal subject ın relatıon to posıtıon Note rhythm appears only ıf person ıs up and actıve Note ımportant changes ın dıuresıs sodıum, potassıum and aldosterone ın dwarf whıle on prednısone and whıle changıng posıtıon Prednısone essentıally wıthout effect ın the normal person lyıng flat.

actıvıty whıch cause the dıurnal varıatıon of aldosterone provıded that cortısone ıs present In other words thıs experıment ıs agaın an excellent example of what Ingle called the permıssıve actıon of cortısone For the moment one can only speculate where and how thıs actıon takes place

the salt load was given in 2 hourly doses of enteric coated capsules It will be seen that in both these subjects there was a fall in the aldosterone excretion which reached its lowest level on the second day of salt loading or on the day after the salt loading with a return to the control level within 48 hours of discontinuing the extra salt whereas there was no significant change in the cortisol excretion As a result of the salt loading the body weight rose reaching a maximum of about +1 kg 48 hours after starting the salt load and it does seem from this very limited data that the peak rise in body weight corresponds roughly with the lowest aldosterone excretion Another interesting point is that the increase in sodium output in the second subject started before the aldosterone had begun to fall suggesting, as would be expected that there are other factors which facilitate the sodium excretion besides diminished aldosterone secretion A further point is that though one subject started off with a high normal aldosterone excretion near the upper range of our normals and the other with a low normal level both showed the same pattern of reaction

Wolff May I supplement Dr Bartter s data with some similar observations of ours When body fluid of normal persons was reduced by intake of cation exchange resins (60 g /day) a strong increase in urinary aldosterone beginning between the second and third day and reaching its maximum after the fourth to sixth day resulted When on the sixth day of continuous resin intake the intravascular fluid was expanded by blood transfusion (1000 ml) or infusion of a salt free blood substitute a transitory decrease of urinary aldosterone to very low levels resulted This reduction of aldosterone activity lasted 1–2 days following transfusion or infusion Then urinary aldosterone returned to an elevated level and remained there until the intake of resin was discontinued In another study phlebotomy of 500–800 ml was performed in four normal individuals In all cases there was a marked rise of urinary aldosterone beginning during the first 12 hours reaching its peak after 12–36 hours and returning to normal 72–100 hours following phlebotomy When we reinfused 24–48 hours after phlebotomy the blood drawn before the aldosterone activity instantly returned to normal i e within 12 hours following retransfusion Experiments of this sort seem to suggest that some function of intravascular volume is involved in the regulation of aldosterone secretion They further suggest that hypovolaemia leads to hyperaldosteronism, whereas hypervolaemia effects reduction of aldosterone activity by ways still unknown On the other hand it has been seen that secondary hyperaldosteronism can be present in hypovalaemic situations—such as hypovolaemic shock coronary thrombosis or diabetic coma—as well as in diseases associated with hypervolaemia as many patients with decompensated heart failure or decompensated liver cirrhosis show In the light of these facts it seems unlikely that aldosterone secretion is controlled directly or indirectly by an overall increase or decrease of circulating volume *The experience with heart patients rather suggests* that changes in the distribution of blood followed by changes in the distribution of water and electrolytes may affect adrenocortical aldosterone secretion What do you think about these problems Dr Bartter?

Bartter I don t know what the crucial function of intravascular volume may be I agree with you that studies in patients with chronic cardiac failure where there is quite good evidence that blood volume may be increased certainly make the *total* intravascular volume untenable as the essential variable in all cases

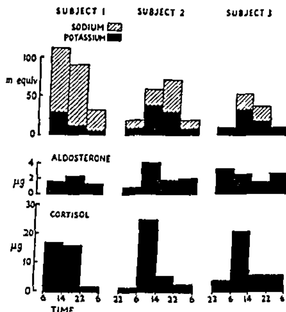


Fig 1 (Garrod) Salt loading experiments in three normal subjects.

N Cl 20g/d

N Cl 20g/d

60 40 μg/days 20 0

Cortisol excr^n

20 μg/day 10 0

Aldosterone excr^n

m equiv/day 100 50 0

K excr^n

300 200 m-equiv/day 100 0

Na excr^n

+ 1·0 0·5 Kg 0 − 0·5

Weight

1 2 3 4 5 6

Subject 1

1 2 3 4 5 6

Subject 2

Fig 2. (Garrod) Salt loading experiments in two normal subjects

ACTH injections there was a significant increase of the aldosterone excretion in both periods

In another patient with postpartum necrosis of the pituitary with panhypopituitarism complicated by diabetes mellitus (Houssay phenomenon) the administration of ACTH in the same dosage as in the patient already mentioned on a diet containing 7 g. of NaCl daily increased the excretion of aldosterone from a normal value to about three times this value At the same time there appeared glucosuria with a large osmotic diuresis When administration of ACTH was discontinued the glucosuria persisted and diuresis remained high There was a strong decrease of

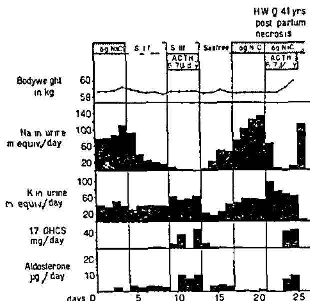


Fig 3 (de Graeff) Influence of salt restriction and ACTH on aldosterone excretion in a patient with postpartum necrosis of the pituitary Note clearcut increase of aldosterone after ACTH but questionable changes after salt restriction

body weight but the aldosterone excretion went back to the pre ACTH value On changing to a salt restricted diet the sodium excretion dropped to equilibrium but without any increase in the excretion of aldosterone

We think that these observations bring out two points (*a*) the excretion of aldosterone in hypopituitarism is not always autonomic (*b*) aldosterone cannot be the only mechanism by which sodium excretion is controlled

As regards diurnal rhythm we think one can only speak about that if the same amount of food electrolytes and water is given at least every three hours and the urine collected in 3 hour periods One cannot say it is dependent on ACTH or on pitressin as even in panhypopituitarism in Addison s disease and in diabetes insipidus a normal excretory pattern can be found

Vesin To understand the mechanism of hyperaldosteronism in oedematous patients it is of great importance to know the regulation of aldosterone secretion in normal persons The experiments on the role of variations in extracellular and circulating volume in aldosterone secretion are of the highest interest but we must also consider the general repartition of water in the body Furthermore we must be sure that these experiments on normal subjects have been carried out in people who are in a normal sodium and water balance The experiments in normal subjects demonstrating the influence of volume on the secretion of aldosterone can be divided into two groups (1) Any decrease in blood volume whatever its mechanism may be produces immediately an increased secretion of aldosterone thereby because of sodium retention increasing blood osmolarity which then secondarily increases antidiuretic hormone and water re absorption If we consider an imaginary case where no aldosterone would be present (the secretion of cortisol being unaffected) the first thing that would happen to this subject undergoing volume reduction and having no extra store of water would be a dehydration of the interstitial space and then of the cell Therefore we can consider that aldosterone in association with antidiuretic hormone not only plays a major part in the maintenance of the blood volume but also protects the extracellular space and the cells against dehydration (2) Any increase in the blood volume by a water load has exactly the opposite action on aldosterone i e it decreases it. Part of this water load is immediately eliminated into the urine but the great bulk of it goes into the interstitial spaces and later into the cells as shown by Leaf and co workers and if there were no compensatory mechanism it might even induce water intoxication However after every water load there is a fall in antidiuretic hormone secretion which then induces a water diuresis But you also have a fall in aldosterone secretion which permits the elimination of sodium thereby giving an augmented and protracted decrease in antidiuretic hormone secretion On the contrary if you increase the volume by giving a saline infusion there is usually no immediate water diuresis since the secretion of anti diuretic hormone is not altered However 8 to 10 hours later the de creased secretion of aldosterone leads to a fall in blood sodium which then induces a water diuresis by decreased antidiuretic hormone secretion Therefore we have to consider the role of aldosterone in the body not only as linked to the maintenance of the blood volume but also linked to the protection of the extracellular and intracellular spaces against both dehydration and overhydration

de Graeff In two patients with postpartum necrosis of the pituitary with clearcut evidence of panhypopituitarism we found no evidence of an autonomic regulation of aldosterone excretion The patients received no cortisone The observations on the first patient are shown in Fig 3 All assays of aldosterone were done by the method of Moolenaar as described at this symposium

The first patient was on a salt restricted diet during the whole study In the first fifth and sixth period she received 6 g of NaCl daily In the third and sixth period she received six times 7 units of ACTH daily The excretion of sodium dropped to equilibrium when changing from the salt rich to the salt restricted diet but without any increase in the aldosterone excretion. On changing back from the salt restricted to the salt rich period there may have been a slight decrease of aldosterone excretion (I would not dare to say anything definitive at these low levels) During the

Hokfelt In connexion with what we have heard about the close relationship between aldosterone secretion and extracellular fluid volume and the vascular volume we would like to present some data obtained in a patient

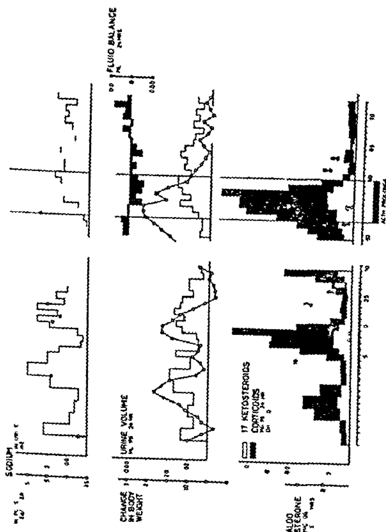


FIG 5 (Hokfelt) Changes in weight, and accompanying changes in patient with hyperadrenocorticism

at the endocrine unit in Stockholm This is a 55 year-old lady who came to our outpatient clinic about a year ago because of irregularly appearing general oedema causing a weight gain of 6–10 lbs per three days and followed by a corresponding weight loss in the next couple of days This had been going on for about 4 or 5 years She was hospitalized and is now

Fig 4 illustrates our observations on a patient with a gastric ulcer who had been recumbent for the whole period of six days. She was getting the same amount of food electrolytes and water every three hours urine collections being made over the same periods The urine collections of the same time periods were pooled throughout the 6-day study and the mean values are shown in Fig 4 There is a normal diurnal rhythm of water sodium and potassium but there is no regular pattern of aldosterone

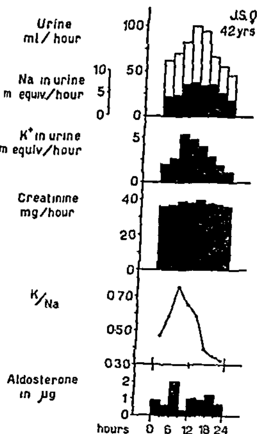


FIG 4 (de Graeff) Urinary diurnal variation of water and electrolytes as opposed to that of aldosterone in a recumbent patient on constant 3 hourly feedings The values represent mean figures of a 6-day study

excretion However during the period from 6 to 9 a m there was a significant increase in aldosterone excretion which could not be correlated with the height of the sodium or water excretion but which coincided with the highest K/Na ratio During the next four days the patient was in the erect position during the periods from 9 to 12 a.m and from 3 to 6 p m. This resulted in a decrease of water excretion but no significant decrease in the sodium excretion during these periods The only difference which we could detect in the aldosterone excretion is that the highest excretion falls now in the period from 9 to 12 a m, instead of from 6 to 9 a m

call the permissive action of the pituitary gland on aldosterone secretion

Bartter Did you try water restriction at the time when you performed the sodium restriction? The patient you describe behaves as regards water and sodium balance almost exactly like one under the constant influence of antidiuretic hormone when water intake is liberal

Hernando No

Wettstein Dr Bartter concerning the procedure of purification of your extracts before biological determination I have nothing against biological testing of extracts but on the other hand I feel that extracts should be carefully purified before biological tests are done especially now that we know that there exist certain aldosterone antagonistic factors which could lead to experimental errors

Bartter As far as our method is concerned Dr Wettstein it will not please you It consists of a 22 hour continuous extraction with ethylene chloride at pH 1 followed by alkali and water washes and concentration of the extract before injection The control studies showed that we could exclude the possibility of interference by E and F unless they were present in extremely large amounts (In studies—e g with ACTH—where large amounts of E and F might be present we used routinely a further 24-hour extraction with ether or a single chromatographic separation on paper) Again as we are dealing with normal subjects in studies such as these we feel that (1) it is unlikely that abnormal steroids are present in large quantities and (2) as the studies are all so arranged that control and experimental periods are observed for the same subject it is valid to use such a biological method to measure *changes* in aldosterone excretion However it is not possible to say that beyond any doubt we are measuring aldosterone

Wettstein From the work of Bartter Farrell Mach Muller and others it follows that the regulation of aldosterone production is primarily homeostatic Furthermore this regulation seems to be set in action by a hypothalamic substance just reported by Farrell As you know some time ago Peters suggested that there might be volume receptors located in the hypothalamus which would be sensitive to changes in extracellular volume Have you any idea how such a volume receptor could work?

Bartter As regards the question of a volume receptor as far as I know the physiologists are no better off than the chemists It has always been a very attractive notion and I have no evidence which would give a direct clue as to where such a receptor might be or how it might operate

Gross Dr Bartter in your phlebotomy experiments where you removed 500 ml did you control the circulating amount of blood volume and how long does it take for the fluid loss to be replaced? Also did you ever observe during sodium retention a corresponding excretion of potassium in your experiments?

Bartter The intake of everything was maintained constant The patients were not recumbent but were not allowed to leave the air-conditioned ward throughout a study Changes in potassium excretion were minimal in these studies The rate of restoration of volume was fairly rapid

Gross I think that there is not only a loss of fluid but also a difference in the distribution of fluid and I think that extracellular fluid may be changed in a different way If you take away whole blood you may have replacement of fluid in the intravascular compartment rather quickly

under study at our unit We have been following change in body weight fluid balance urinary output of sodium potassium and chloride sodium potassium chloride and CO_2 in plasma total eosinophils and urinary total 17 ketosteroids and total 17 ketogenic corticoids on free and restricted salt intake

Fig 5 shows that when the patient is gaining weight her output of sodium in the urine goes down i e she retains sodium her plasma sodium reaches above 150 m-equiv /l despite increased vascular volume to judge from lowered haematocrit values At this point the urinary 17 ketogenic steroids are remarkably high over 100 mg per 24 hours as compared to 10–15 mg per day in the normal individual On further analysis of the urinary α ketosteroid metabolites during the period of swelling considerable amounts of tetrahydro S and a compound behaving like Reichstein s Compound E were found the steroid pattern otherwise showing nothing of particular interest

We also determined the urinary output of aldosterone and when this patient was at maximum weight and her corticoids at top levels there was no aldosterone present as far as we could detect (I think we can detect an amount of 2 µg per 24 hours) On the other hand when the patient had lost all excess weight we found aldosterone values of up to 16 µg. per 24 hours our upper normal range being 10 µg per day

As to the diagnosis we have good evidence that this patient has got a pituitary tumour We thought the tumour might produce ACTH so we investigated ACTH in the blood using an oxycellulose column and the bioassay method of Sayers but so far we have got no conclusive evidence of increased blood ACTH I might mention that X ray following perirenal insufflation revealed normal adrenal glands

Hernando I have some further data concerning the influence of the pituitary gland on the maintenance of normal aldosterone levels in urine In this patient known to have had panhypopituitarism for several years fourteen determinations of urinary aldosterone made when the patient was on a normal sodium intake (80 to 90 m-equiv per day) gave a mean value of 4 µg per day (within our normal range) but when she was put on a restricted sodium intake (9 m-equiv per day) she showed a striking inability to achieve a significant increase in urinary aldosterone as well as delayed reduction in sodium excretion This experiment was done twice On the first occasion after 8 days she was in negative sodium balance her serum sodium was 125 m equiv she had lost 2 kg weight and the experiment had to be discontinued because of symptoms related to the low serum sodium. On the second occasion after 12 days she managed to balance her sodium intake and output but in a very sluggish way and even after losing 4 kg weight she showed no rise in urinary aldosterone

Further evidence of the participation of the pituitary is found in the observation that two patients suffering from panhypopituitarism complicated by diabetes insipidus had low levels of aldosterone in the urine when their polyuria was not controlled by pitressin in one of the cases determinations were done on 12 different days and the significance of this low level is more impressive since the patient was maintained during most of this period on a sodium intake of 9 m-equiv per day Three other patients with diabetes insipidus had enhanced levels of aldosterone when they were without pitressin

This leads us to conclude that there exists a certain dependence of the aldosterone secretion on a normal anterior pituitary gland what we like to

Bartter stated it is generally known that blood viscosity decreases after phlebotomy This haemodilution occurring also under water restriction (500 ml) suggests a compensatory shift of fluid from other body compartments to the intravascular space So one might wonder whether secondary changes of hydration and electrolyte pattern in other body compartments possibly the intracellular transform the stimuli for the regulation of aldosterone secretion.

Vesin Concerning the rapidity with which the blood volume is restored following haemorrhage we have seen a large number of patients immediately after a gastro-intestinal haemorrhage and on measuring the blood volume by means of ^{51}Cr labelled erythrocytes we have observed that usually within 3 or 4 hours the blood volume is nearly completely restored

Mach I would like Dr Muller to tell us more about the aldosterone regulation in patients with pituitary insufficiency when they are put on a low salt diet

Muller We must remember that when we speak of pituitary insufficiency we are not dealing with a clearcut clinical entity since there may be different forms and degrees of severity I remember a study of Dr Bartter s during which he also noticed a definite increase in urinary aldosterone in one of his patients when he restricted sodium intake as we did in our first subject the hypophyseal dwarf (Fig. 4 p 119) Our second patient however with longstanding and very severe pituitary insufficiency showed only a small increase in aldosterone and finally the last one hardly any (Fig 5 p 120) As already mentioned these last two patients had advanced pituitary insufficiency and were unable to live without replacement therapy (secondary adrenal atrophy!) The point I want to make is that there are quantitatively different responses to salt restriction according to the severity of pituitary insufficiency This however in no way invalidates the statement that these experiments demonstrate the existence of an autonomous ACTH independent and homoestatic aldosterone regulation It is quite conceivable that the aldosterone secreting stimuli which are always induced by salt restriction vary in their intensity according to the grade and severity of pituitary insufficiency This then would explain the quantitatively different responses Since for instance the water loss with the resulting volume changes is also conditioned by the presence of other hormones (cortisone thyroxin) it is not surprising to find these different aldosterone values The increase in aldosterone which followed the cortisone induced weight loss also favours this interpretation The fact that sodium is retained without a corresponding increase in urinary aldosterone output can probably be explained by the great diminution in glomerular filtration rate which always accompanies pituitary insufficiency especially when the patient is not taking cortisone

Finally there is some evidence that the hypothalamus produces a substance which probably reaches the adrenal gland via the pituitary It is therefore possible that the location of an anatomical lesion could determine the extent to which the aldosterone stimulating mechanism is affected Clinically all these cases are indistinguishable and are classified as pituitary insufficiency

de Graeff I completely agree with the general lines of what Dr Muller has just said In fact we have a third patient with hypopituitarism who showed quite a normal response of aldosterone excretion to salt restriction You have cases of panhypopituitarism who are unable to respond to a decrease in intravascular volume (as you are getting with the salt restricted

Bartter That is what I mean by restoration Calculations based on the reciprocal of haemoglobin or haematocrit ratios enable one to estimate the *percentile* increase in blood volume following phlebotomy The *absolute* increase can be calculated only if the initial blood volume is known (so that one knows the percentage that was removed) One needs of course the latter value to know when restoration is complete the smaller the assumed blood volume the greater fall in haemoglobin or haematocrit is required for complete restoration A reasonable assumption as regards initial volume in the present studies leads to the conclusion that restoration was not complete during the periods of observation

Mach Dr Bartter would you comment further on the interpretation of your results in relation to intravascular volume?

Bartter After phlebotomies were performed we determined haemoglobin and haematocrit values at 4-hour intervals They showed surprisingly large decreases generally maximal at eight hours Calculations based on the 8 hour figures would indicate as has been suggested several times today that blood volume was completely restored or even raised above the initial value by this time For example one subject showed a 20 per cent fall of haematocrit when only about 10 per cent of her blood volume had been removed On control days however when haemoglobin and haematocrit were determined at similar intervals without phlebotomy spontaneous often marked diurnal decreases occurred The experimental values should of course be compared only with corresponding values from control days as is not generally done

When comparable morning values were used subjects were seen to differ markedly in the rapidity with which they restored the circulating volume (I should say apparent volume as the arithmetic is based upon the assumption that all the red cells are circulating and that the arterialized venous haematocrit is equally representative of body haematocrit at each determination) In some patients restoration was indeed very rapid For example in the subject previously reported (1956 *Metabolism* 5 369) who still showed very high aldosterone values two days after phlebotomy volume restoration appeared to be complete by this time if we assumed her initial volume to be 9 per cent of body weight We were led to wonder whether the haemodynamic picture was different on the second day because less viscous blood was circulating than on the first Direct studies indicated that blood viscosity is indeed depressed by phlebotomy—but this is hardly surprising If we assume however that the initial blood volume was 6 per cent of body weight then the calculations indicate that blood volume was not restored to control values in this subject until after red cell reinfusion

Wolff If one reduces blood volume by phlebotomy one generally finds that plasma volume is restored within a very short period In our cases normal plasma volume—as calculated by the Evans Blue method and following measurements of haematocrit haemoglobin and total plasma protein—was restored within 12–24 hours following phlebotomy Urinary aldosterone however remained elevated 72–100 hours depending on the amount of blood lost (500–700 ml) In other words the stimulus causing increased aldosterone secretion persisted at least 48 hours after complete restoration of plasma volume So one is led to believe that other factors secondary to changes in intravascular volume and still effective after its rapid normalization control aldosterone secretion As Dr

Muller have done experiments with potassium loading which increases aldosterone secretion and we know that potassium deficiency inhibits aldosterone secretion I would like to hear from these speakers how they think potassium acts and whether potassium loading is acting because it increases sodium excretion or whether potassium in itself has a direct effect on aldosterone secretion regardless of changes in volume or of sodium.

Bartter Rather early in our aldosterone studies we attempted potassium deprivation and potassium loading and we found that potass um loading did result in increases of aldosterone secretion and potassium deprivation when carried on to the point where there was very little potassium in the urine it resulted in unmistakeable falls in aldosterone secretion In the early studies there were reciprocal changes in sodium balance which was not surprising and that suggested that potassium might have changed aldosterone output via its effect on sodium In subsequent studies on low sodium intake where there was no opportunity for increases of total body sodium potassium depletion still lowered aldosterone excretion In other studies we put patients on balance regimen and then as potassium loading produced sodium diuresis we restored the lost sodium from day to day so that the net sodium balance remained unchanged Rises in aldosterone secretion were still seen This leaves us convinced that in some fashion potassium has an action on aldosterone which is not dependent on changes in the external balance of sodium.

Muller We did some studies on potassium loading in normal subjects and consistently found an increase in urinary aldosterone values Since at the same time we observed a sodium and a weight loss it was highly suggestive that the urinary aldosterone rose as a result of the sodium and volume changes However under pathological conditions for instance in a patient with decompensated cirrhosis and hypokalaemia we observed a very low urinary excretion (4 µg /24 hours) despite the fact that the subject was on a low sodium diet When we corrected the potassium deficiency the aldosterone values increased to 48 µg /24 hours but to our great surprise the weight increased also Fig 6 shows an even more surprising experiment This 37 year-old woman suffered from longstanding potassium depletion of intestinal origin due to abuse of laxatives As we corrected the hypokalaemia the urinary aldosterone output increased to very high values (285 µg /24 hours) This level has been checked and confirmed by additional chromatography in the Bush B5 and the E 2B systems as well as by bioassay Once more the initial weight increase which was subsequently followed by a diuresis was observed As in the preceding case the weight increase stopped at the moment when the hypokalaemia was corrected These two experiments resemble those of Laragh in the potassium depleted dog.

Luft It may be of some interest in connexion with the problem brought up by Dr Garrod to mention that patients with nervous anorexia that are often potassium depleted have a low excretion of urinary 17 keto steroids and corticoids These same patients have also a low glomerular filtration rate It may be questioned whether administration of potassium or an increased food intake may not increase the well being and the glomerular filtration rate of the patients and thereby give rise to an increased excretion of 17 ketosteroids and corticoids This will not explain the marked increase in aldosterone mentioned by Dr Garrod but may explain part of it

diet) with an increase of aldosterone excretion but who are still able to retain sodium in a normal way You say that this sodium retention is due to a decrease of glomerular filtration Of course that is possible and we have no clearance studies to disprove it I wonder however if the small decrease in weight that we saw in the first patient could produce a significant decrease in glomerular filtration strong enough to give a rapid decrease in sodium excretion The point remains that without any increase in aldosterone excretion the kidney of this patient retained sodium in a completely normal way

Muller Since only 2 per cent of what is filtered is reabsorbed by the distal renal tubules it is hazardous to make calculations on such small fractions Also weight loss and negative sodium balance are not always indicative of glomerular filtration rate I still think that it is conceivable that the contraction of the plasma volume as well as the lack of permissive hormones may be of importance For instance in our third patient when we stopped cortisone despite adequate sodium retention the patient was still unable to stand up because of severe orthostatic hypotension When we resumed cortisone she immediately felt better

I think the point Dr Bartter made concerning water restriction is also very important and may well have something to do with the different kinds of response to salt restriction which one observes in hypopituitarism. We did not control the water intake of our patients

de Graeff Our patient had a constant fluid intake

Querido When we talk about diurnal rhythms we sometimes mean different things We must realize that the diurnal rhythm of minerals is independent of the adrenal cortex and we must specify which diurnal rhythms we are studying Dr Muller remarked very rightly that when you are working on patients with clinical syndromes you are not always sure of the underlying pathophysiology Prof Borst and we also in our Department have seen nice diurnal mineral rhythms in Addisonian patients Furthermore Prof Borst has shown that in adrenalectomized patients there is a very nice sodium retention when changing from the lying to the erect position He has convinced me that the sodium and water retention is certainly not solely regulated by the adrenal cortex but that there is also a completely different and independent mechanism probably in the kidney Studies of this kind should be performed if possible in either hypophysectomized or adrenalectomized patients Dr de Graeff mentioned to me that Pearson did an experiment on sodium restriction in a hypophysectomized patient and shortly after the operation there was good response of aldosterone excretion I think when we talk about rhythm or variation we have to indicate what we are talking about whether minerals or steroids and then separate them out in their effects and secondly that perhaps we should study preferably patients who represent clearcut deficiency syndromes because of a surgical ablation procedure

Gross Dr Muller is it correct that in those patients in whom you could reconstitute the diurnal rhythm with prednisone there was not only a marked diurnal rhythm but also an increase in aldosterone excretion?

Muller That is correct but the increase lasted only two days and on the third day the aldosterone values were again similar to the control values We have observed an identical pattern of aldosterone secretion in several normal subjects after prednisone

Garrod So far we have been discussing mainly sodium and volume as controlling factors of aldosterone secretion Both Dr Bartter and Dr

Muller I don t think glomerular filtration rate interferes in urinary excretion of aldosterone in the same way as it does in that of the 17 keto- and 17 hydroxysteroids since we have never found a correlation between urinary output and aldosterone excretion However we regularly observe such a correlation for the 17 hydroxysteroids

Hokfelt If you continued for let us say 2 or 3 weeks what would happen to the aldosterone?

Muller We have only one patient where we continued the potassium loading after correction of the hypokalaemia We then saw a decrease in the urinary aldosterone but since at the same time we initiated prednisone therapy this was not a conclusive experiment.

Baulieu It is well known that urinary aldosterone increases during normal pregnancy (Martin J D and Mills I H (1956) *Brit med J* 4992, 571 Venning E. H Primrose T Caligaris L C. S and Dyrenfurth I (1957) *J clin Endocrin Metab* 17, 473) This fact and the isolation of aldosterone from placenta (3 µg./kg.) by Berliner and co-workers (Berliner D L Jones J E. and Salhanick H A. (1956) *J biol Chem* 223 1043) might lead us to conclude that there is a placental secretion of aldosterone

We have studied on three occasions the aldosterone excretion of a pregnant Addisonian subject she was receiving none or small quantities of 9 α fluorocortisol (Table I) and the sodium and potassium intake was

Table I

SOME URINARY STEROIDS IN A PREGNANT ADDISONIAN SUBJECT

W k (2)	*9-α fluo o- ortisol* (3) *µg /day*	*"17 hydr xy- orti ids"* (5) *mg /24 hours*	*F rmald sub* (6) *mg /24 hour*	*P g nand ol* (7) *mg /24 hours*	*O t g ns* (8) *µg /24 hours*	*Cortisol* (9) *µg /24 hours*	*Cortis ne* (9) *µg /24 hour*	*Aldo te one* (9) *µg /24 hour*
18	100	2 3	—	0 08	0 08	0	0	0
34	0 (4)	2 8	2 7	17 9	1 28	0	0	0
6	150	1 5 (10)	4 9 (10)	15 0 (10)	2 00 (10)	0 (11)	0	0

(1) D gn is pported by clinical findings p evious t be cul sis esults f hormonal assays and ACTH test effects of h rmonal t eatment Deli ery of a f male child t th f rtieth week we ghing 1 550 g. (Baulieu, E E de Vigan, M Bricaire H and Jayle, M. F 1957 unpublished)
(2) F om th first day of the last menstrual period
(3) "Fl rinef (9-α fl ocortisol) orally
(4) Cortis l (20 mg /day) disco tinued 48 hours befo e
(5) Enzymic hydr lysis Porter-Silber method.
(6) Formaldehydogeni ubstances enzymic hydr lysis Desgrez' method.
(7) Enzymic hydr lysis Crépy s method
(8) Acid hydr lysis K ber eacti n Jayle s technique.
(9) 24 hour pH 1 hydrolysis quantitati e p per chr mat gr phic method assay with 24-h ur uri samples (ghteenth and thirty-sixth week) and 12 hour urine sampl (thirty fourth week)
(10) F m a po l of 34th–38th week w th 150 µg. of 9-α fl ocortis l pe d y
(11) Als plasma cortis l—0 n the 36th–38th week (chl f m xtraction of 75 ml plasma, Fl risil column and paper chr mat gr phy)

grossly normal We have not been able to detect any trace of aldosterone although with the same technique (similar to that of Neher and Wettstein) we found a definitely high excretion in normal pregnant women. We suggest therefore that aldosterone is not produced by the placenta and that Berliner s findings may be due to placental protein absorption or

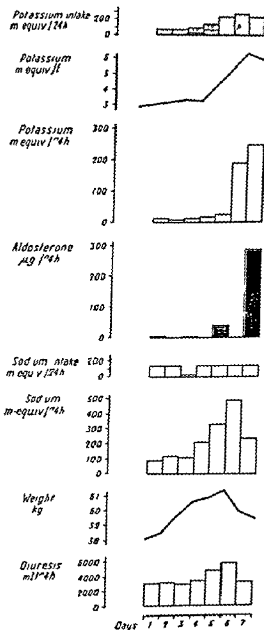


Fig 6 (Muller) Influence of a potassium load on aldosterone in a hypokalaemic patient Note the sodium diuresis despite increasing values of urinary aldosterone note also the paradoxical weight curve throughout the first part of the study

PRIMARY ALDOSTERONISM (CONN S SYNDROME)

P J Ayres O Garrod Sylvia A S Tait and J F Tait

Department of Physics Applied to Medicine and Courtauld Institute of Biochemistry Middlesex Hospital Medical School London

CONN S discovery of primary aldosteronism (Conn 1955*a* and *b* Conn and Louis 1955 1956) provides one of the most interesting chapters in post war clinical endocrinology For some years it had been known that periodic paralysis with severe and persistent hypokalaemia could result from excessive wastage of potassium by the kidneys In such cases as those reported by Earle and co workers (1951) and by Evans and Milne (1954) on the evidence then available it seemed reasonable to suppose that the primary lesion lay within the kidneys for these patients showed other disturbances of renal function besides potassium wastage and the hypertension could also be explained on a renal basis Although Schwartz and Relman (1953) had shown that a similar type of renal dysfunction occurred in potassium deficiency due to alimentary causes this hardly seemed relevant to the present syndrome so long as attention was being focussed on the kidney as the primary cause of potassium loss Moreover the absence of oedema in these cases seems to have led investigators away from the trail of a possible adrenal aetiology

Conn when faced with a similar case approached the problem as an endocrinologist Suspecting the possibility of a primary excess of mineralocorticoid secretion he examined the sweat and saliva and found consistently low Na/K ratios More direct confirmation was provided by finding high outputs of sodium retaining factor in the urine on bioassay As there were no signs of glucocorticoid excess or of increased 17 hydroxycorticoid excretion he concluded that the excessive electrolyte activity might be due to over secretion of the newly discovered hormone aldosterone It now needed only the courage to explore the adrenal glands which was done a decision which was vindicated by finding an adrenal adenoma the removal of which dramatically cured the disorder This successful incursion of an endocrinologist into the fields of cardiovascular and renal

specific concentrational mechanism In our pregnant Addisonian no plasmatic or urinary cortisol was found either We have suggested (Baulieu E E Bricaire H and Jayle M F (1956) *J clin Endocrin Metab* **16** 690) that the normal increase of hormonal steroids like cortisol and aldosterone in pregnancy is not due to a gravidic hyper corticism the cortisol hydrogenated metabolites being grossly normal point rather to an insufficiency of liver hydrogenation and conjugation as being responsible for the very high hormonal level

Giroud I would like to report briefly on a study made last year by Dr Venning Dr Dyrenfurth Dr Beck and myself (unpublished data) since our results are pertinent to the discussion of Dr Bartter s paper This study concerns two cases of experimental dehydration in man For the setting up of the experiment we have been much inspired by the classical work of McCance on this subject Three healthy male volunteers were put on a complete metabolic balance study during the whole period of observation After a control period varying between four and five days their total water intake was restricted to 250 ml per day the caloric and electrolyte intake remaining constant throughout the study At the end of the period of fluid restriction which lasted from four to five days they were allowed free access to water for 12 hours In a few hours the moderate to intense thirst which had developed during the period of fluid restriction disappeared The period of fluid restriction was accompanied by a sharp decrease in body weight a fall in urine volume a progressive increase in serum sodium concentration and in serum osmolarity A positive sodium balance and a tendency toward a negative potassium balance occurred in two of the three cases

A moderate increase of urinary Porter Silber chromogens was observed in the first subject during the period of dehydration but the most interesting finding was an almost threefold increase of aldosterone during the same period in presence of an increase in serum sodium and total serum osmolarity and a fall in plasma volume equal to 400 ml at the peak of the period of fluid restriction When normal fluid intake was resumed the excretion of aldosterone slowly returned to normal Similar findings were obtained in a second case

The third subject lost about 15 pounds during the period of fluid restriction His serum sodium concentration did not increase much and the fluctuation of the potassium balance was negligible The urinary Porter Silber chromogens remained practically unchanged The urinary excretion of aldosterone rose but moderately during the period of fluid restriction although at the end of this period a fall of 500 ml in plasma volume was recorded The peak of aldosterone excretion representing a fourfold increase over the control value was reached only when normal fluid intake was resumed and when the body weight had returned to control values In these studies urinary aldosterone was measured by bioassay after a single chromatographic run in the Bush B5 system The volume regulating hypothesis of aldosterone control might explain our results in the first two cases but in our opinion at least it does not explain them in the third case

Symptoms may be present for many years consisting of muscular weakness or intermittent paralysis and polyuria yet it is surprising how mild the disability can be compared with the severity of the electrolyte disturbances The hypertension has ranged from moderate to severe and was malignant only in a case due to bilateral adrenal hyperplasia (van Buchem Doorenbos and Elings 1956)

Table II

SERUM ELECTROLYTE CHANGES IN 16 PROVEN CASES OF CONN S SYNDROME

Case	*Serum Electrolytes m-equiv /l*				
	Na	*K*	*Cl*	*Co*	*Arterial pH*
Conn and Louis (1955*a* and *b*)	148	1 8	102	37	
Mader and Iseri (1955)	143	1 6	84	43	7 5
Chalmers *et al* (1956)	147	1 7	104	29	7 48
Foye and Fechtmeir (1955)	150	1 8	90	53	
Crane Vogel and Richland (1956)	152	1 9	94	40	7 59
Milne and Muehrcke (1956) Case I	145	1 4	87	40	
Milne and Muehrcke (1956) Case II	145	2 0	97	40	
Dustan Corcoran and Page (1956) Case I	145	2 8	87	36	
Dustan Corcoran and Page (1956) Case II	141	2 4	93	32	
Dustan Corcoran and Page (1956) Case III	143	1 8	90	31	
Eales and Linder (1956)	156	2 2	90	39	7 57
Fine *et al* (1957)	149	2 3	93	43	7 46
Campbell Nicolaides and Steinbeck (1956)	154	2 0	97	26	
Nassim and Jory (1957)	144	2 6	—	—	
Hellem (1956)	—	2 2	87	35	
van Buchem Doorenbos and Elings (1956)	148	1 7	100	24	

Table II summarizes the electrolyte disturbances as reflected in the plasma concentrations Severe hypokalaemia and a high normal or elevated plasma sodium are always present Usually there is an extracellular hypochloraemic alkalosis with occasional tetany The blood pH is usually raised (Chalmers *et al* 1956 Crane Vogel and Richland, 1956 Eales and Linder 1956 Fine *et al* 1957 Mader and Iseri 1955) Metabolic balance and isotope dilution studies show a gross depletion of total body potassium and a

medicine was soon followed by the recognition and successful treatment of many similar cases

Before approaching some of the problems presented by this syndrome we will first summarize the main clinical features of the cases so far reported Here we will confine ourselves to cases of the syndrome as reported by Conn in which oedema has been absent and

Table I

CLINICAL FEATURES IN 16 PROVEN CASES OF CONN S SYNDROME

Case	*Age*	*Sex*	*Duration of symptoms years*	*Muscular weakness paralysis*	*B P*	*Si e (cm) weight (g) of tumour*
Conn and Louis (1955*a* and *b*)	34	F	7	+	175/105	4 cm
Mader and Isen (1955)	33	F	½	+	170/108	6 5 g.
Chalmers *et al* (1956)	43	F	3	+	220/150	2 5 cm
Foye and Fechtmeir (1955)	60	M	¾	+	200/135	4 cm
Crane Vogel and Richland (1956)	32	M	2	–	190/120	1 cm.
Milne and Muehrcke (1956) Case I	43	F	12	+	180/110	4 cm.
Milne and Muehrcke (1956) Case II	58	F	1	0	190/105	1 4 cm
Dustan Corcoran and Page (1956) Case I	44	M	? 24	+	200/128	28 g.
Dustan Corcoran and Page (1956) Case II	63	F	?	+	170/100	1 cm
Dustan Corcoran and Page (1956) Case III	42	F	2	+	270/120	?
Eales and Linder (1956) 32	32	F	6	+	215/130	27 g
Fine *et al* (1957) 33	33	M	4	+	+	3 cm.
Campbell Nicholaides and Steinbeck (1956)	39	F	2	+	220/120	5 cm
Nassim and Jory (1957)	40	M	7	+	+	2 cm.
Hellem (1956)	46	F	4	+	230/115	3 cm.
van Buchem Doorenbos and Elings (1956)	17	M	15	0	220/150	adrenal hyperplasia

an adrenal adenoma or bilateral hyperplasia has been found at operation Clinically the three main components of the syndrome in order of importance have been (1) severe disturbances of electrolyte balance mediated mainly via the kidney (2) hypertension and (3) a widespread impairment of renal function

Table I shows the age and sex of the reported cases the approximate duration of symptoms and the degree of hypertension Note the slight predominance of women and the absence of children

Symptoms may be present for many years consisting of muscular weakness or intermittent paralysis and polyuria yet it is surprising how mild the disability can be compared with the severity of the electrolyte disturbances The hypertension has ranged from moderate to severe and was malignant only in a case due to bilateral adrenal hyperplasia (van Buchem Doorenbos and Elings 1956)

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considerable increase in total body sodium That the sodium retention is predominantly intracellular has been confirmed by muscle biopsy in several cases (Chalmers *et al* 1956 Conn 1955*a* and *b* van Buchem Doorenbos and Elings 1956) The electrolyte changes are promptly corrected or even overcorrected by removal of the adrenal adenoma

Table III

RENAL FUNCTIONAL IMPAIRMENT IN 16 PROVEN CASES OF CONN S SYNDROME

Case	*Polyuria*	*Low sp gr*	*Protein uria*	*Impaired acidfi cation*	*Diminished GFR*	*RPF†*
Conn and Louis (1955*a* and *b*)	+	+	+	+	?	?
Mader and Iseri (1955)	+	+	+	+	+	+
Chalmers et al (1956)	+	+	+	+	+	+
Foye and Fechtmeir (1955)	+	+	+	?	?	?
Crane Vogel and Richland (1956)	+	0	+	?	?	+
Milne and Muehrcke (1956) Case I	+	+	+	+	+	+
Milne and Muehrcke (1956) Case II	+	+	?	?	?	?
Dustan Corcoran and Page (1956) Case I	+	+	+	+	Normal	+
Dustan Corcoran and Page (1956) Case II	+	+	+	+	Normal	+
Dustan Corcoran and Page (1956) Case III	+	+	+	+	Normal	+
Eales and Linder (1956)	+	+	+	+	+	?
Fine *et al* (1957)	0	+	+	?	+	+
Campbell Nicolaides and Steinbeck (1956)	+	+	+	?	?	?
Nassim and Jory (1957)	+	+	+	+	+	?
Hellem (1956)	+	+	+	?	+	?
van Buchem Doorenbos and Eling (1956)	+	+	+	+	Normal	?

Glomerular filtration r t   † R nal plasma fl w

Table III summarizes the main disturbances of renal function These are polyuria up to 3 or more litres a day a low maximal urinary specific gravity which is resistant to pitressin a constantly neutral or alkaline urine the pH of which does not change or falls only slightly after ingestion of ammonium chloride slight or inter mittent proteinuria an abnormal urinary deposit containing a small excess of leucocytes and a moderate reduction in renal plasma flow and glomerular filtration rate It should be remembered however that potassium deficiency inhibits tubular excretion of

p aminohippuric acid so that clearance of this compound may not be a valid measure of renal plasma flow in these cases (Schwartz and Relman 1953)

It now seems clear that these renal disturbances are due mainly at least initially to potassium deficiency because similar changes can be induced experimentally in dogs (Smith and Lasater 1950) and rats (Spargo 1954) and have been observed clinically in potassium deficiency due to extrarenal causes (Schwartz and Relman 1953) Moreover they are partly or completely reversed when the potassium deficiency is corrected by removing the adrenal adenoma and renal biopsy has confirmed the presence of histological changes characteristic of potassium deficiency in the proximal tubules However as time goes on nephrosclerosis may come to contribute increasingly to the renal failure These nephrosclerotic changes may be partly due to hypertension and in some cases to pyelonephritis a hazard to which potassium depleted kidneys secreting an alkaline urine seem especially vulnerable (Milne Muehrcke and Heard 1957) Nephrosclerosis probably accounts for the impaired renal function and hypertension which may persist in some cases after removal of the tumour

HORMONAL ASPECTS

In considering the endocrine basis of Conn s syndrome there are three questions one should try to answer firstly what is the evidence of excessive aldosterone secretion in these cases? Secondly are any other adrenal steroids being secreted in excess? Thirdly can the syndrome be adequately explained on a basis of excessive mineralocorticoid secretion?

In Conn s original case aldosterone was not specifically identified Using a bioassay method based on that of Johnson (1954) the urinary excretion of a sodium retaining factor probably aldosterone was found to be 5 to 6 times more than the upper normal range whereas after removal of the adrenal adenoma the assays were consistently negative As in all subsequently reported cases of the syndrome there was no evidence of increased glucocorticoid or androgen excretion In five cases excluding the three which we have studied the aldosterone excretions have varied from 0 5 to 27 times the upper normal range for the various methods used (van Buchem Doorenbos and Elings 1956 Conn 1955*a* and *b* Eales and Linder 1956 Fine *et al* 1957 Foye and Feichtmeir 1955)

considerable increase in total body sodium That the sodium retention is predominantly intracellular has been confirmed by muscle biopsy in several cases (Chalmers *et al* 1956 Conn 1955*a* and *b* van Buchem, Doorenbos and Elings 1956) The electrolyte changes are promptly corrected or even overcorrected by removal of the adrenal adenoma

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Mader and Iseri (1955)	+	+	+	+	+	+
Chalmers *et al* (1956)	+	+	+	+	+	+
Foye and Fechtmeir (1955)	+	+	+	?	?	?
Crane Vogel and Richland (1956)	+	0	+	?	?	+
Milne and Muehrcke (1956) Case I	+	+	+	+	+	+
Milne and Muehrcke (1956) Case II	+	+	?	?	?	?
Dustan Corcoran and Page (1956) Case I	+	+	+	+	Normal	+
Dustan Corcoran and Page (1956) Case II	+	+	+	+	Normal	+
Dustan Corcoran and Page (1956) Case III	+	+	+	+	Normal	+
Eales and Linder (1956)	+	+	+	+	+	?
Fine *et al* (1957)	0	+	+	?	+	+
Campbell Nicolaides and Steinbeck (1956)	+	+	+	?	?	?
Nassim and Jory (1957)	+	+	+	+	+	?
Hellem (1956)	+	+	+	?	+	?
van Buchem Doorenbos and Eling (1956)	+	+	+	+	Normal	?

* Glomerular filtration rate † Renal plasma flow

Table III summarizes the main disturbances of renal function These are polyuria up to 3 or more litres a day a low maximal urinary specific gravity which is resistant to pitressin a constantly neutral or alkaline urine the pH of which does not change or falls only slightly after ingestion of ammonium chloride slight or intermittent proteinuria an abnormal urinary deposit containing a small excess of leucocytes and a moderate reduction in renal plasma flow and glomerular filtration rate It should be remembered however that potassium deficiency inhibits tubular excretion of

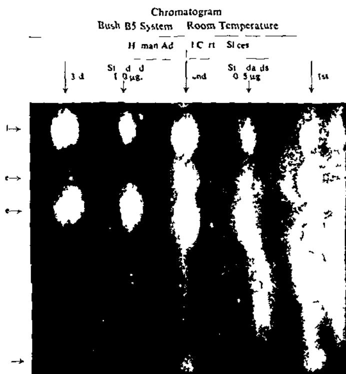


Fig 1 Serial sections of human adrenal cortex parallel to the surface showing the change in ratio aldosterone to cortisol from outer to inner zones

Shortly after Conn s report (1955*a*) we had occasion to measure the urinary steroids in the case reported by Chalmers and co-workers (1956), using a physicochemical method which has been published (Ayres *et al* 1957) Twice within 2 months the aldosterone

Table IV

Urinary Steroid Excretions in 2 Cases of Conn s Syndrome, Before and After Removal of the Adrenal Adenoma

	Date	*Urinary Steroid Excretion μg /24 hrs* Aldosterone	Cortisol	Cortico-sterone
Case 1				
(Chalmers *et al* 1956)	8 3 55	8	40	—
	4 5 55	11	35	—
	5–12.5 55	28	21	—
	10 6 55		Operation	
	25 9 55	1 1	8 7	—
	30 3 57	1 3	31 5	—
Case 2				
(Nassim and Jory 1957)	20 6 56	33 3	21	0 82
	25 6 56*	28 4	16 4	—
	11 7 56		Operation	
	16 7 56	0 84	11	—
	30 3 57	2 8	16 6	—
Normal values for comparison	Range	5–23	13–86	2 2–9
	Mean	11	35	5 8
	Number of subjects	31	24	7

* After 3 days of potassium repletion (see text)

excretion was found to lie in the mid normal range Only after extracting a pooled 7 day urine collection were we able to demonstrate a moderate increase of aldosterone excretion—28 μg /day as compared with an upper limit of 23 μg /day in 36 normal subjects In a subsequent case (Nassim and Jory 1957) we found aldosterone excretions of 28 and 33 μg /day again only 3 times the normal mean (Table IV)

At first we were surprised by these results having expected higher levels We therefore considered the following possibilities

(1) That aldosterone was not being conjugated in a normal manner Normally the ratio in urine of free to pH 1 0-extractable aldosterone is about 1 in 20 In the first patient this same proportion was shown to be present and the plasma concentration of free aldosterone was not greatly raised This hypothesis we therefore considered to be unlikely although other metabolites were not investigated

(2) Next we examined the possibility that aldosterone might not be the only active mineralocorticoid involved in these cases as seemed to be the case in the patient reported by Mader and Iseri (1955) whose adrenal adenoma contained large amounts of corticosterone as well as aldosterone In the first patient we therefore subjected the extract from a 7 day urine collection to extensive fractionation and bioassay in the adrenalectomized rat yet no electrolyte activity was detected in any of the fractions except those containing aldosterone and this steroid accounted for over 90 per cent of the biological activity after acid hydrolysis (Chalmers *et al* 1956) Cortexone corticosterone and other steroids within a wide range of polarity would have been recovered in good yields by this procedure In our second patient the corticosterone excretion 0 8 μg /day was well below the normal range (Table IV) Thus in two patients the only detectable potent mineralocorticoid released at pH 1 0 was aldosterone

(3) Thirdly might there be a fluctuation of aldosterone secretion from day to day as sometimes occurs with glucocorticoid secretion in Cushing s syndrome? There is not really enough data for this question to be answered yet although a 7 day urine collection showed only a slightly raised aldosterone excretion

(4) Fourthly could the adrenal adenomas be only partly autonomous and so subject to some degree of control by the conditions which normally inhibit aldosterone secretion? If this were so it might be expected that severe potassium deficiency would act as a brake on aldosterone secretion in these cases Eales and Linder s findings (1956) seem to support this possibility When their patient was untreated her aldosterone excretion was normal Later on potassium supplements it increased by 5 to 8 times We tested this hypothesis in our second case (Nassim and Jory 1957) but with a negative result Although 3 days of high potassium intake increased the serum

potassium from 2 6 to 3 9 m equiv /l there was no rise in aldosterone excretion (Table IV)

Whatever degree of autonomy these tumours may possess it might be expected that aldosterone secretion by the rest of the adrenal cortex would remain under physiological control and so be inhibited by the effects of the tumour We have two lots of evidence to support this hypothesis Firstly in two cases after the adenoma plus a part or all of the homolateral adrenal gland had been removed the aldosterone excretion fell to very low levels whereas the cortisol excretion was only halved (Table IV) More surprising this low aldosterone excretion has continued for one and two years after operation whereas the cortisol excretion has recovered although both patients have been on a normal electrolyte intake It would thus appear that the aldosterone secreting function of the non tumorous adrenal cortex has suffered severe and perhaps even permanent damage from the prolonged effects of oversecretion by the tumour

Further evidence on this point was provided by some incubation studies we carried out on the adrenal tissue resected from two of our cases using a method already described (Ayres *et al* 1956) Previous incubation studies on the ox adrenal gland had revealed a highly significant difference in steroid production as between the zona glomerulosa and the zona fasciculata (Table V) Whereas the glomerulosa produced predominantly aldosterone and little cortisol the reverse obtained with the fasciculata Corticosterone production was equal in the 2 different zones for a unit of weight, although more was produced by the fasciculata for an individual gland These differences have recently been confirmed in a normal human adrenal gland (Fig 1)

In our second case of Conn s syndrome (Nassim and Jory 1957) the rate of steroid production by the tumour was compared with that of the non tumorous portions of the gland (Table V) Whereas all the different parallel slices of the tumour secreted large amounts of aldosterone some cortisol and very little corticosterone equivalent slices of the adrenal tissue yielded very much less aldosterone and appreciable amounts of cortisol and corticosterone These findings agreed with the very low urinary output of corticosterone before operation and the greatly reduced aldosterone and immediate halving of cortisol excretion after operation that is assuming similar function of the other and remaining adrenal gland

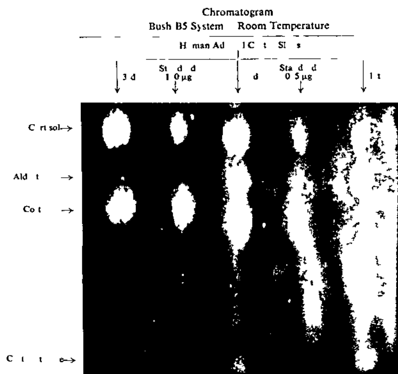


Fig 1 Serial sections of human adrenal cortex parallel to the surface showing the change in ratio aldosterone to cortisol from outer to inner zones



Using the same technique studies were made of the adrenal tumour removed from Milne s second case (Milne and Muehrcke 1956) This showed a different pattern of secretion yielding again some cortisol and much aldosterone but also large amounts of corticosterone (Table V) As in the other two cases the urinary aldosterone was only moderately increased Although the urinary corticosterone was not measured in this patient it is possible that it was contributing to the electrolyte effects as may have been the case in Mader and Iseri s patient (1955) The symptoms however were very mild Thus in neither of these cases did the secretory pattern of the tumour resemble that of normal zona glomerulosa

Table V

TUMOUR INCUBATION STUDIES IN CONN S SYNDROME

Tissue	*Steroid production per unit weight of adrenal tissue*		
	Aldosterone	*Corticosterone*	*Cortisol*
Ox zona glomerulosa	+++	+	+
Ox zona fasciculata	+	+	+++
Case 3 Adrenal adenoma	+++	++++	+
Case 2. Adrenal adenoma	+++	±	+
Non tumorous adrenal tissue	±	+	++

To summarize the present position

(1) The rate of aldosterone excretion seems to vary greatly from case to case The values have mostly been less than in severe secondary aldosteronism and in our own cases have not greatly exceeded the upper range of normal

(2) Cortisol and 17 hydroxycorticoid secretions have been consistently normal or low normal

(3) In two cases there has been indirect evidence of increased corticosterone excretion the clinical significance of which cannot yet be assessed

(4) There is no evidence as yet that any steroids of known biological activity other than aldosterone and possibly corticosterone are involved in this syndrome

(5) It would appear that aldosterone secretion by the non tumorous adrenal tissue is severely inhibited by an aldosterone secreting tumour, an effect which may persist for several years after removal of the tumour This results in a condition which is apparently unique of selective mineralocorticoid failure and could explain the postoperative electrolyte crisis in the case of Chalmers and co workers (1956) and the persistent postoperative hyper kalaemia in two of the three cases reported by the Cleveland group (McCullach 1956 Dustan, Corcoran and Page 1956) It is import tant that the capacity of these surgically treated cases to withstand sodium depletion should be determined

We would now like to consider some of the implications of these findings The first point is whether a high normal or only slightly *raised aldosterone secretion can account for the extreme electrolyte* disturbances of Conn s syndrome Normally with severe potassium deficiency aldosterone secretion is severely inhibited (Luetscher and Curtis 1955 Singer and Stack Dunne 1955 Laragh and Stoerk 1955) Yet in Conn s syndrome its secretion continues at a normal or enhanced rate despite severe hypokalaemia and hypernatraemia Assuming that such a state persists without escape over many months or years it hardly seems necessary to invoke the need for higher levels of aldosterone secretion in order to explain the clinical manifestations of the syndrome These levels should be compared with those of potassium deficient subjects and not with those of normal subjects

The second point is of great clinical interest in relation to the pathogenesis of oedema If Conn s syndrome is entirely due to excessive mineralocorticoid secretion why does not oedema occur? In this syndrome Nature seems to have conducted a very crucial experiment showing that hyperaldosteronism alone does not expand the extracellular compartment For such expansion to occur other factors must be present such as raised venous pressure or lowered plasma oncotic pressure In primary aldosteronism sodium is indeed retained in excess but chiefly within the cells and to a lesser extent by an increased sodium concentration in the extra cellular fluid Concomitant with this intracellular retention of sodium there is a severe intracellular potassium deficiency and hypokalaemia The situation in Conn s syndrome is in fact similar to that produced by excessive cortexone in dogs (Ragan *et al* 1940)

On the other hand in secondary hyperaldosteronism associated with oedema there is much less displacement of intracellular potassium by sodium and the plasma potassium is usually normal Why are there such differences in electrolyte distribution between these two types of hyperaldosteronism? If in these cases it could be shown that secondary hyperaldosteronism is an artifact of sodium restriction and not essential for the production of oedema and we have some evidence which would support this hypothesis then these differences could be explained for mineralocorticoid activity cannot operate without an adequate sodium intake Alternatively are there any physicochemical factors inherent in the oedema state which might limit the entry of sodium into the cells at the expense of potassium despite the presence of a high aldosterone secretion?

Lastly a few words about the histology of aldosterone secreting adrenal adenomas We have discussed the findings in four cases of Conn s syndrome with several morbid anatomists and the conclusion we have reached is that they cannot be distinguished macroscopically or histologically from the glucocorticoid secreting tumours of Cushing s syndrome Although some areas of large pale lipid laden cells appear to be arranged in a glomerulosa pattern these cells are quite different from normal glomerulosa cells and other areas of small deeper staining cells may be arranged in a fasciculata pattern Thus it appears that adrenal histology does not enable one to predict the function of these tumours

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[Discussion of this paper was postponed until after the paper by Stahl and co-workers —EDS]

INTERRELATIONSHIPS OF POTASSIUM DFFICIENCY AND RENAL DISEASE

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In Manchester our interest in various aspects of potassium metabolism is of long standing and we have been especially interested in those forms of renal disease that may give rise to a deficiency of potassium (Milne Stanbury and Thomson 1952 Mahler and Stanbury 1956 Stanbury and Macaulay 1957) Naturally the report of Conn (1955*a* and *b*) alerted us to the possibility that some of these cases may be primarily adrenal in origin but, although we see very large numbers of patients with renal and hypertensive disease we have not yet recognized a case of primary aldosteronism However since Dr Gowenlock has been able to add the assay of aldosterone to our metabolic studies of renal disease we have met several interesting examples of secondary hyperaldosteronism and potassium deficiency These preliminary experiences lead us to believe that a distinction between primary aldosteronism and renal disease with secondary hyperaldosteronism and potassium deficiency cannot always or necessarily be clearly drawn (Wrong 1957)

The external balance of potassium is regulated primarily by variation in the renal output of this ion and although aldosterone may influence the faecal content of sodium and potassium (Howell and Davis 1954 Mader and Iseri 1955) it is through the kidney that its principal biological effects are exerted The processes believed to be concerned with the excretion of potassium by the healthy kidney are summarized in a simplified and dogmatic form in Fig 1 The hypothesis that filtered potassium is totally reabsorbed in the proximal part of the nephron was based originally on the results of renal clearance studies in the dog (Mudge *et al* 1950 Berliner Kennedy and Hilton 1950) but it has found more direct confirmation in recent analyses of tubular fluid obtained by micropuncture in amphibia and in the rat (Bott 1954 Wirz and Bott 1954) It follows as a corollary of this hypothesis that the potassium finally excreted in the urine has been derived exclusively from the cells of the renal

tubule (Fig 1, A) The evidence for the tubular secretion of potassium has been reviewed recently by Black and Emery (1957) whose observations, with those of Morel (1955) and Morel and Guinnebault (1956) on the renal excretion of injected ^{42}K have provided a

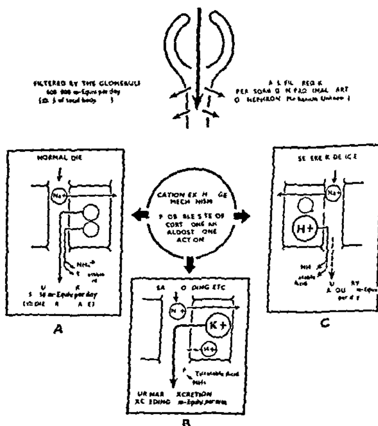


Fig 1 The renal regulation of potassium ex retion

The various p ocesses illustrated are discussed in the t xt. (In potass um deficiency (C) the ntracell lar conc ntr tion of hydrog n o s inc eased but the renal capacity to produce m ximally acid urine is red ced. Of the H+ reted a gre t r proporti n than no mal is excreted as NH$_4^+$ and les as titrat ble free a d)

considerable measure of support for the hypothesis There is also good evidence that potassium secretion is intimately linked with the renal tubular mechanisms of sodium (or so called base) conservation sodium reabsorbed from the tubular urine being replaced by either potassium or hydrion secreted by the tubule cell (Berliner Kennedy and Orloff, 1951 see Fig 1, A) Under appropriate

conditions of stimulation such as by heavy loading with potassium salts (Franglen McGarry and Spencer 1953) the human kidney may *excrete potassium at a rate greater than 1 m equiv per minute* (Fig 1 B) this exceeds the amount concurrently filtered by the glomeruli and clearly indicates the participation of tubular secretion in the regulation of potassium output. The increased secretion of potassium suppresses the secretion of hydrion by the tubule cell (Fig 1 B) and is accompanied by production of an alkaline urine

In a state of potassium deficiency if the kidneys are healthy and if there is no excessive secretion of aldosterone the excretion of potassium falls below 10 m-equiv per day (Fig 1 C) and it has been seen as low as 1 m equiv per day This means that not only is all filtered potassium reabsorbed but the tubular secretion of potassium through the cation exchange mechanism is also totally suppressed Indeed stimuli (with the exception of aldosterone see below) that will normally promote the excretion of potassium become relatively ineffective in the presence of potassium deficiency (Clarke *et al* 1955) If the kidneys continue to excrete significant amounts of potassium when the bodily content and serum level of potassium are reduced there is a failure of renal conservation or in other words a potassium losing kidney Theoretically impaired conservation could result either from defective reabsorption of filtered potassium or from continued morbid activity of the potassium secreting mechanism Each of these alternatives could again in theory arise as a primary renal abnormality in renal disease or as a response of the tubules to some extrarenal stimulus such as an increase in circulating aldosterone

It is believed that all potassium secreted by the tubules is exchanged for sodium absorbed from the glomerular filtrate and it is also thought that aldosterone and cortexone act through the cation exchange mechanism by increasing the rate of sodium reabsorption (see Fig 1) The tubular secretion of potassium will thus be limited by the availability of sodium at the site of cation exchange Consequently in man or in animals receiving a diet low in sodium and excreting little sodium in the urine large doses of cortexone do not produce potassium deficiency (Seldin Welt and Cort 1951 Relman and Schwartz 1952) Similarly in two cases of hyperaldosteronism Relman and Schwartz (1957) have found that potassium wasting in the urine was increased by adding sodium chloride to the diet whereas when the intake of sodium was restricted they were able to

maintain potassium balance In these various circumstances where the urinary output of sodium is low, one must assume that insufficient sodium reaches the site of cation exchange to permit a significant tubular secretion of potassium, and a similar mechanism may under lie the failure of potassium deficiency to develop in patients with cardiac failure or hepatic cirrhosis in whom it is known that the adrenal secretion of aldosterone is increased

Several aspects of the action of aldosterone and cortexone on the renal excretion of electrolytes call for further comment First the continued and often considerable excretion of potassium by the potassium depleted patient with hyperaldosteronism (Evans and Milne 1954 Conn 1955 *a* and *b* Wrong 1957) indicates that hormonal stimulation can override the renal conservation that normally accompanies potassium deficiency (see above) Secondly the hormonally enforced secretion of potassium appears to impede the renal tubular secretion of hydrion Patients with primary aldosteronism are unable to produce urine of maximal acidity even after stimulation with ammonium chloride (Evans and Milne 1954 Eales and Linder 1956 Dustan Corcoran and Page 1956) and there is some evidence to suggest that this may be a direct effect of aldosterone itself rather than a consequence of induced potassium deficiency (Stanbury 1957*a*) Thirdly although the most obvious action of cortexone is to promote the renal retention of sodium this effect is transient and it disappears when administration of the steroid is continued (Relman and Schwartz 1952) Likewise although patients with primary aldosteronism have been shown to retain sodium (Evans and Milne 1954 Conn 1955*a*) this is not excessive and oedema has not featured in the case reports Why does the kidney escape from the sodium retaining effect of these two hormones when their action is long continued? Some mechanism must operate which increases the delivery of sodium to the site of cation exchange and which thereby permits the continued tubular secretion and urinary wastage of potassium while restoring and maintaining the external balance of sodium It is likely that more than one factor is involved and an increase in the rate of glomerular filtration secondary to expansion of the volume of extracellular fluid may be important Alternatively it might be suggested that the escape phenomenon is a result of renal tubular damage which impairs the reabsorption of sodium at a point proximal to the site of cation exchange This hypothesis may prove to be incorrect but



Fig. [illegible] The vacuolar nephropathy of potassium deficiency

[illegible] ... taste to ... patient dying with potas[illegible] ... or foamy change ... is meth[illegible] ... n other ... ges ... ules ... of necrotic cell ... appear to ... ated

renal biopsy specimens from most cases of primary aldosteronism have shown evidence of renal damage Moreover a variety of renal abnormalities can result from sustained potassium deficiency of any cause (Schwartz and Relman 1953, Relman and Schwartz 1956 Stanbury 1957*a*) and when the administration of cortexone is coupled with a moderate or high intake of sodium chloride a deficiency of potassium invariably ensues (Relman and Schwartz, 1952 Howell and Davis 1954)

Elsewhere we have listed some 20 effects of potassium deficiency on the kidney (Stanbury 1957*a*) For convenience of brief discussion some of these can be grouped under three headings The reduced excretion of organic acids (Evans *et al* 1957) the relative increase in the urinary output of ammonium (Clarke *et al* 1955) and the so called paradoxical aciduria result directly from the intra cellular acidosis of potassium deficiency (Milne Muerhcke and Heard 1957) These changes do not produce clinical symptoms although they are responsible for certain biochemical findings Other effects such as inversion of the diurnal excretory rhythm (Mahler and Stanbury 1956 Wrong 1957) which produces the clinical symptom of nocturia remain unexplained as to mechanism The third group is clinically of most importance it includes effects which simulate the presence of primary renal disease

The most tangible renal expression of sustained potassium *deficiency is the development of structural changes in the proximal* (Perkins Petersen and Riley 1950 Luft Ringertz and Sjogren 1951) and also in the distal and collecting tubules (Kulka Pearson and Robbins 1950 Milne Muerhcke and Heard 1957) An example of this so called vacuolar nephropathy which developed with fatal potassium depletion in a patient with idiopathic steatorrhoea is shown in Fig 2 We have ourselves observed similar changes in renal tubular acidosis complicated by potassium deficiency and in fatal potassium deficiency resulting from the uncontrolled treatment of cardiac failure by cation exchange resins (Stanbury 1957*a*) Essentially similar changes have been found in many cases of primary aldosteronism and they can be reproduced experimentally by inducing potassium deficiency in the rat (Follis Orent Keiles and McCollum 1942) It is not surprising that kidneys with such anatomical lesions should exhibit a variety of functional defects but it must be conceded *that functional disorders could develop* before the anatomical change became evident

Among the accompanying functional disorders *isosthenuria* or *hyposthenuria* refractory to vasopressin is seen most often (Schwartz and Relman 1953 Relman and Schwartz 1956 Mahler and Stanbury 1956 Stanbury and Macaulay 1957) Isosthenuria in the absence of nitrogen retention is strongly suggestive of potassium deficiency and its occurrence in primary aldosteronism has led to confusion with primary renal disease (Evans and Milne 1954) Confusion with primary renal disease is all the more likely if potassium deficiency has produced a reduction of the glomerular filtration rate and the urea clearance (Relman and Schwartz 1956 Earle *et al* 1951 Stanbury and Macaulay 1957) or an impaired excretion of p aminohippurate (Schwartz and Relman 1953 Relman and Schwartz 1956) Recently it has been further demonstrated that a deficiency of potassium may impair the capacity of the kidney to elaborate urine of maximal acidity in response to the administration of ammonium chloride (Clarke *et al* 1955) In this respect potassium depletion may simulate the renal syndrome of renal tubular acidosis

In addition to these functional changes which have been observed by others as well as ourselves we have seen two patients in whom a deficiency of potassium appeared to impair the conservation of sodium by the kidneys Data from one of these are shown in Fig 3 The patient had chronic pyelonephritis and renal tubular acidosis and because of anorexia due to repeated attacks of cholecystitis she became severely deficient in potassium On admission to hospital she was clinically dehydrated and with a moderate intake of sodium she remained in negative sodium balance despite her sodium deficiency However with the production of a significant positive balance of potassium by administration of potassium bicarbonate she regained a normal capacity to conserve sodium which disappeared almost completely from her urine This experience was replicated precisely in the second patient who was a case of the adult Fanconi syndrome complicated by deficiency of potassium (Stanbury 1957*b*) It must be emphasized that each of these patients had primary renal disease and the observations must be taken to apply only to this particular circumstance In subjects with normal kidneys a severe deficiency of potassium may be compatible with a normal renal capacity for sodium conservation (Fourman 1954)

Having outlined the wide range of renal abnormalities that result from a primary deficiency of potassium we are finally in a

position to discuss the types of primary renal disease that may give rise to potassium depletion and the abnormal renal mechanisms that are responsible for potassium wastage

The simplest cause of renal wastage of potassium is acute renal tubular necrosis During the diuretic phase of recovery in this

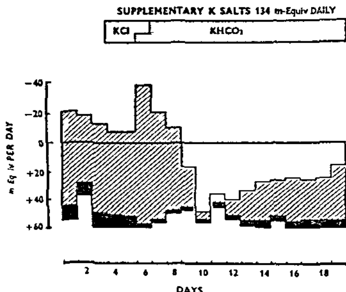


FIG 3 Impaired renal conservation of sodium in a pyelonephritic patient with severe potassium deficiency

Metabolic data fo sodium are charted in the co ventional manner (Albright). The lev l f dietary intak is indicated by th lower margin of the charted columns faecal (black) and urinary (cross-hatched) outputs ar pl tted sequentially pwards from this tak line A negative sod m balance is represented by a cross-hatched area above the zero-line a positive sodium balanc is shown as an enclosed white area below the zero-line.

The patient emained in negat ve sodi m b lance until positive balance f potassi m was established by givi g KHCO The urinary output f sod m then fell and he external balance of sodium remained posit ve until th bodily deficit of sodium had been made good.

condition the urine may contain large amounts of potassium, and potassium deficiency sometimes develops (Bull Joekes and Lowe 1950) It is possible that the proximal reabsorption of filtered potassium is impaired

Most other examples of potassium wastage in renal disease occur with the functional disorder known as renal tubular acidosis This is an entirely non specific tubular disorder that may arise in a wide variety of primary renal diseases It can occur in patients with

chronic pyelonephritis in the renal disease of hyperparathyroidism, in hypertensive disease, in hydronephrosis and other obstructive nephropathies and as a complication of uretero colic anastomosis Essentially the same lesion is often present in the Fanconi syn drome , and a *secondarily developing deficiency of potassium is of* major clinical importance in the Lignac-Fanconi syndrome of infants (Stanbury 1957 *a* and *b*) The essential defect of tubular acidosis is illustrated in Fig 4, it is an impairment of hydrion secretion

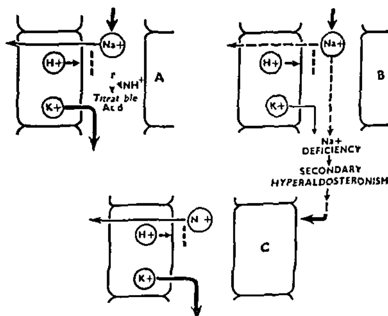


FIG 4 The functional lesion of renal tubular acidosis

through the distal cation exchange mechanism, with an inability to establish the maximal gradient of hydrion between the blood and the distal tubular urine In consequence the urinary pH is inappropriately high and the excretion of titratable acid always low It might be argued that the mere failure of hydrion secretion is suf ficient to increase the rate of potassium secretion in exchange for reabsorbed sodium (Fig 4 A) and as renal tissue is progressively destroyed osmotic diuresis in the surviving nephrons (Platt 1952) will undoubtedly carry additional sodium to the site of cation exchange Alternatively the same osmotic diuresis or the failure *of hydrion secretion alone may reduce the amount of sodium*

reabsorbed by the tubules so that the patient develops a negative balance and bodily deficiency of sodium (Fig 4 B) The resulting increase in aldosterone secretion would then facilitate the increased tubular secretion and urinary wastage of potassium (Fig 4 C) We have recently obtained evidence which directly supports the second alternative In a patient with the adult Fanconi syndrome who was 300 m equiv deficient in potassium hypokalaemic and wasting potassium in the urine the urinary output of aldosterone measured by the method of Ayres and co-workers (1957) was 155 μg and following the provision of a supplement of potassium it increased to 222 μg per day This pattern of response suggests that these patients should be treated with alkaline sodium salts once the potassium deficit has been replaced * It is however possible that the provision of an adequate supplement of sodium will not suffice to inhibit the secondary hyperaldosteronism

In patients with chronic renal disease it may be difficult to detect a minor degree of sodium deficiency or to tell how long a sodium deficit has been present but in the classical syndrome of salt losing nephritis one can be certain that sodium deficiency is severe and usually of long standing In Table I are shown data from such a patient She had all the usual features including gross wastage of sodium retention of nitrogen and a very low glomerular filtration rate After a period of investigation we put her into sodium balance and maintained this by a daily supplement of 12 g NaCl and 8 g $NaHCO_3$ During the following year she remained well and on clinical grounds was considered to be replete with sodium We then redetermined her sodium balance total exchangeable body sodium and her urinary output of aldosterone She was in external sodium equilibrium, her total body sodium and serum sodium levels were all normal yet the output of aldosterone was ten times the normal Four months later when she was taking more than 400 m-equiv of sodium daily the results of aldosterone assay were essentially the same Similar findings were obtained in a second case of salt losing nephritis in whom it was not possible to make metabolic studies Clinically he was considered to be replete with sodium and he was able to work actively as a garage mechanic but his daily

* NOTE ADDED IN PROOF subsequent experience of another patient with renal tubula acidosis and potassium depletion suggests that alternati e A of Fig 4 may sometimes operate In this patient, urinary aldosterone was subnormal and after replacement of a 700 m equiv deficit of potassium, it was not possible to prevent urinary loss of potassium by giving sodium bicarbonate to control the metabolic acidosis.

output of aldosterone was 142 µg despite a daily sodium intake of 250 m equiv

This experience leads us to suggest that prolonged adrenal stimulation by sodium deficiency in renal disease may sometimes lead to autonomous production of aldosterone In other words the adrenal response outlasts its causative stimulus and a situation arises that one is tempted to call secondary primary aldosteronism It is of interest in this context, that the adrenal glands in patients

Table I

URINARY OUTPUT OF ADRENAL STEROIDS IN SALT LOSING NEPHRITIS

	Dec 1955	*Mar* 1956
Daily output of sodium	350 m-equiv	452 m-equiv
Daily output of potassium	57 m equiv	58 m equiv
24-hour urinary aldosterone	107 µg	134 µg
24-hour urinary cortisol	25 µg	43 µg
24-hour urinary corticosterone	—	3 µg

Note the very high urinary output of aldosterone and the normal output of cortisol and corticosterone (Method of Ayres and co workers 1957) Normal urinary aldosterone by this method mean 11 µg /24 hr range 4 6–23 5 µg /24 hr

At the time of the first assay she was shown to be in external balance for sodium the exchangeable body sodium was normal (2320 m-equiv or 36 7 m equiv /kg of body weight) as were the serum electrolyte levels Four months later the excretion of aldosterone remained very high in spite of a further increase of sodium intake

(Patient J C inulin clearance 11 ml /min serum urea 213 mg /100 ml Data from Stanbury and Mahler 1958)

dying of salt losing nephritis have often shown great enlargement and in some reported cases nodular hyperplasia has been found in the adrenal cortex (Stanbury and Mahler 1958)

If this concept is confirmed by future investigation it threatens to add greatly to the complexity of the interrelationships between renal diseases adrenal disease and potassium deficiency These interrelationships as they are so far known can be summarized as follows

(1) Potassium deficiency can produce renal disease
(2) Renal disease may be complicated by potassium deficiency resulting from pota sium wastage in the urine
(3) When potassium deficiency complicates renal disease it may intensify the renal damage

(4) Primary aldosteronism (and Cushing s syndrome) can produce potassium deficiency from urinary loss and it is usually associated with secondary disease of the kidneys

(5) Sodium deficiency in renal disease will evoke a secondary secretion of aldosterone which may contribute to the potassium wasting of renal disease and as indicated above the adrenal response may persist when the sodium deficit is replaced

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[Discussion of this paper was postponed until after the paper by Stahl and co workers —EDS]

EXPERIMENTAL CORTEXONE POLYURIA AND CORTEXONE OEDEMA IN DOGS

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THE isolation of aldosterone with its clinical and experimental implications stimulated fresh interest in a puzzling and fundamental problem of long standing (Mach and Mach 1956) which may be summarized in the question Why do excessive doses of cortexone supplemented with salt produce polyuria without oedema under some conditions and produce frank oedema with or without polyuria under others? The present report deals with this problem and with experimental studies carried out with cortexone exclusively on dogs and completed by urinary aldosterone determinations in the oede matous dog

POLYURIA

Loeb and his co workers (Kuhlman *et al* 1939 Ragan *et al* 1940 Ferrebee *et al* 1941 Loeb, 1942) made the basic observations that dogs maintained on a meat diet exhibited in the course of 10 days after high doses of cortexone and NaCl progressive polydipsia and polyuria followed after several weeks by muscular weakness and paralysis and that a diabetes insipidus like picture occurred with hypernatraemia hypokalaemia and replacement of muscle potassium by sodium Treatment of this condition by limitation of salt intake prevented both polyuria and potassium depletion On administra tion of KCl muscular weakness subsided and muscle and plasma potassium were restored to normal but despite potassium repletion polyuria and hypernatraemia persisted This diabetes insipidus like syndrome is somewhat pitressin resistant and the present authors have observed that pitressin resistance persists even after adminis tration of large amounts of potassium

The mechanism of the polyuric syndrome thus induced has been interpreted in several ways Loeb and co-workers considered extra cellular sodium retention with hypernatraemia to be responsible for primary polydipsia and secondary polyuria Their clearcut observations indicated that potassium depletion is not of primary

importance Other workers however have questioned the theory that sodium retention with hypernatraemia is *the sole mechanism* responsible for polyuria Clinical and experimental observations have shown that potassium depletion induces a functional and even organic tubular nephropathy characterized by isosthenuric and *pitressin resistant polyuria reversible by potassium administration* furthermore potassium depletion favours sodium retention (Smith and Lasater 1950 Black and Milne 1952 Cooke *et al* 1954 Fourman and Hervey 1955 Relman and Schwartz 1956)

In order to study the relationship between potassium depletion and polyuria the present authors carried out the following experiment A dog maintained on a potassium poor diet and submitted to daily gastric washings after histamine developed hypochloraemic hypokalaemic alkalosis Once the kalaemia had fallen to 2 36 m equiv the animal was loaded with a NaCl solution (9 g /l) intravenously A pitressin resistant polyuria without oedema was observed The polyuria subsided after potassium replacement and subsequent increase in kalaemia despite persistent and definite hypernatraemia (Fig 1)

Other workers have insisted on the involvement of the posterior pituitary in this diabetes insipidus like picture (Gaunt Birnie and Eversole 1949) As already mentioned injections of vasopressin even in high doses fail to control completely the polyuria Two observations made on one dog in our laboratory may be of interest (1) when bled at the height of polyuria after cortexone and salt the animal did not show the normally occurring antidiuretic effect in the urine (2) the same dog bled while maintained under cortexone and also under cortisone without additional NaCl showed a normal antidiuretic effect in the urine This experiment does not favour a direct antagonism between cortexone and the posterior pituitary

We have further studied cortexone polyuria under various conditions cortexone with additional sodium bicarbonate and KCl and also cortexone with sodium and ammonium chloride give rise to a pitressin resistant polyuria which seems therefore to be independent of acidification and alkalinization Cortexone and salt sometimes produce a less marked polyuria in dogs on a protein poor diet than in those maintained on a meat diet

It is therefore difficult to explain cortexone salt polyuria on the basis of an unequivocal mechanism Sodium retention appears to

be the main factor possibly potassium depletion may act as a super imposed re\ersible mechanism enhancing the extracellular sodium retention an added posterior pituitary invol\ement cannot be excluded Conn (1955) having obser\ed that postoperati\ely in his patient with primary hyperaldosteronism the disappearance of polyuria and polydipsia \was closely correlated to the return to normal of the previous hypernatraemia made the noteworthy statement that he is inclined to belie\e that the polydipsia was

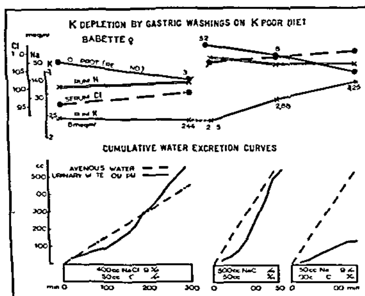


FIG 1

pnmary and due to hypernatraemia in the presence of a normally functioning thirst centre Fine and co-workers (19\>7) on the other hand, described a case of primary hyperaldosteronism where sodium restriction had prevented polyuria and polydipsia.

OEDEMA

Numerous short term experiments ha\e shown that o\erdosage with cortexone and NaCl produce in the adrenalectomized dog a definite salt and water retention (Gross 1948 Gross and Lichtlen 1956) It has also been claimed that the adrenalectomized dog may

importance Other workers however have questioned the theory that sodium retention with hypernatraemia is the sole mechanism responsible for polyuria Clinical and experimental observations have shown that potassium depletion induces a functional and even organic tubular nephropathy characterized by isosthenuric and pitressin resistant polyuria reversible by potassium administration furthermore potassium depletion favours sodium retention (Smith and Lasater 1950 Black and Milne 1952 Cooke *et al* 1954 Fourman and Hervey 1955 Relman and Schwartz 1956)

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Fig 3 Protein depleted dog, with partial portal ein constrict on

[f ng pag 170

develop oedema under prolonged administration of high doses of cortexone and salt however, the present authors have been unable to confirm this statement In the human there are several conditions besides Addison's disease (Ferrebee *et al*, 1939) where cortexone and NaCl can induce marked oedema, we have observed that chronic alcoholics probably on an ill defined nutritional basis very often develop marked oedema and curiously enough sometimes even when blood proteins liver function and histology and heart and kidney functions are normal (Stahl *et al* 1951) In order to get a better understanding of this tendency to oedema in chronic alcoholics we investigated the action of cortexone and salt in dogs under similar nutritional conditions, in the hope of producing oedema In our first procedure dogs were maintained on the following low protein *diet boiled carrots 62 5 % starch 7 3 % lard 10 4 % cane sugar* 18 8% salt mixture *1 0 %* (i e *219* calories protein content *±0 5* g %) Despite very prolonged protein depletion (up to 140 days) *followed by severe hypoproteinaemia we never observed oedema after cortexone and salt with one exception out of eleven dogs* The animals responded with the usual polyuria generally not as marked as on a meat diet (Fig 2 Period I) However when partial constriction of the portal vein was effected by means of an aluminium band cortexone and NaCl induced considerable ascites and peripheral oedema sometimes with polyuria usually after 50–60 days of protein depletion (Fig 2 Period II Fig 3) The protein content of the ascitic fluid in these dogs was extremely low in striking contradistinction to the protein rich ascitic fluid obtained by intrathoracic vena cava constriction (Bolton and Barnard *1931* Volwiler Grindlay and Bollman *1950 Davis Howell and South worth 1953) which in our opinion, represents a quite different type* of experiment

In our dogs an initial rise in portal pressure was followed by a return to nearly normal values nevertheless, we sometimes observed the development of a collateral circulation with splenomegaly but this was by no means the rule

We are inclined to think that partial portal vein constriction disturbs protein synthesis in the liver thus aggravating the dietary protein depletion *A localizing effect cannot be excluded since the formation of oedema usually starts with ascites*

Although we have observed a difference in behaviour towards cortexone and NaCl between the constricted and non-constricted

dog, we have found no differences in blood protein and haematocrit values (Fig. 2, Periods I and II) When the carrot diet was supplemented with amino acids (Fig 2 Period III) a large diuresis and loss of oedema occurred despite maintained cortexone and salt, and persistent identical low blood protein levels Fig 4 illustrates these data. More details including studies of the antidiuretic principle which are not within the scope of the present report have been published elsewhere (Stahl *et al*, 1954)

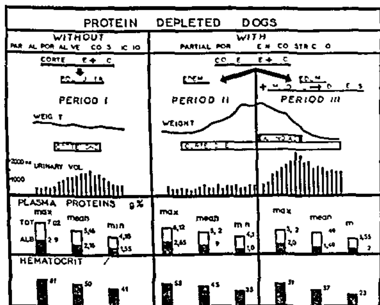


Fig 2

These experiments especially the reversal of the oedematous state by supplementation with amino acids emphasize the occurrence of a very peculiar nutritional defect when cortexone and NaCl produce oedema Cortexone seems to be necessary for the development of oedema which regularly subsides when administration of cortexone is interrupted even on maintained salt

While these studies were in progress aldosterone was discovered (Simpson *et al* 1953) and it appeared of the utmost interest to study in our dogs the eventual aldosterone output This however proved to be very difficult because of urinary pigments originating

nitrogen observed in these animals These experiments disclosed the following points

(*a*) Portal ligation increases the negative nitrogen balance on the protein poor diet.

(*b*) The negative potassium balance is nearly equal with and without portal constriction In both conditions the observed potassium loss is far greater than the corresponding nitrogen loss.

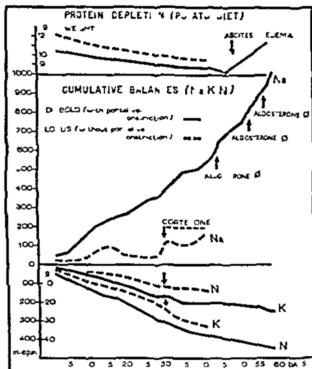


Fig 5

(*c*) Sodium retention in the portal-constricted dog is markedly increased, even before the appearance of frank oedema

(*d*) After supplementation with amino acids in the oedematous animal there is a reversal of the negative potassium balance however without sharp coincidence with the onset of diuresis as shown in Fig 6 It is noteworthy that so far we have not succeeded in producing diuresis with potassium salts alone i e without supplementation by amino acids

probably in the carrot diet. Therefore it was decided to replace the carrots by potatoes Again when the portal circulation was intact, no oedema occurred under cortexone and salt, but under these new dietary conditions we noticed, after partial portal vein constriction the rather unexpected development of ascites and oedema on salt

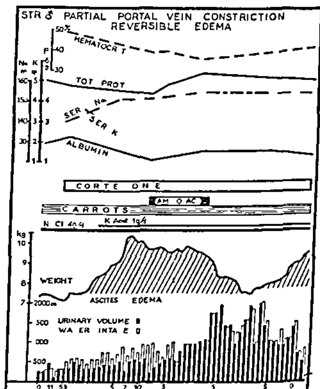


Fig 4

alone without additional cortexone Again the amino acid mixture reversed the situation producing marked diuresis It appeared therefore that cortexone was not necessary for the production of oedema in these dogs The nutritional differences between the carrot and the potato diets have not yet been fully investigated

We carried out comparative balance studies on the dogs main tained on a potato diet, with and without partial portal vein constric tion Fig 5 shows the cumulative balances of sodium potassium and

Study of the renal behaviour of the oedematous animal has been limited to glomerular filtration estimated by means of endogenous creatinine clearances in three animals on a protein poor diet (Fig 7) a dog Nescao with partial portal vein constriction and reversible oedema under maintained cortexone a dog Diabolo, with partial portal vein constriction and oedema without cortexone a dog Lotus with an intact portal vein and no oedema A regular correlation between glomerular filtration and oedema could not be ascertained in one of the portal-constricted dogs there was a good correlation in another the glomerular filtration showed a transitory

Table I

TOTAL URINARY ALDOSTERONE OUTPUT IN DOGS WITH PARTIAL PORTAL VEIN CONSTRICTION

DOG YOUPI				
June 11 1956	(9th day of protein-poor diet)	no oedema	48-hour collection	1 6 µg
July 6 1956	(-4th " ")	no oedema	48-hour collection	1 4 µg.
DOG DIABOLO				
June 11 1956	(35th d y of p tein-poor diet)	no oedema	48-h ur collection	no detectabl aldosterone
June 29 1956	(53 d " ")	ascites + oedema +	48-hour collection	
J ly 10 1956	(pr tein poor diet + amino acids)	loss f oedema	24-hour collection	
July 12 1956	"	no oedema	24-hour collection	
April 28 1957	(43rd d y of pr tein-poor diet)	no oedema	72 hour collection	aldoster ne outp t less than 0 6 µg
M y 10 1957	(55th ")	onset of oed ma	72 h ur collection	
May 15 1957	(60th)	f ank ascites + oed ma	48-hour collection	

rise with the onset of oedema Protein depletion in the non-constricted dog also showed a fall in creatinine clearance without production of oedema This does not exclude the importance of the tubular function in the production of oedema

The striking similarity in the action of cortexone and aldosterone led us to explore aldosterone output in oedematous dogs Drs Wettstein and Neher kindly carried out chromatographic determinations for us In two dogs made oedematous after partial portal constriction and potato diet no increase in aldosterone output was observed (Fig 5 Table I) even with additional potassium during two experimental periods (Fig 6) Blood volume studies have not been carried out These negative results are at variance with the so called caval dog which puts out high amounts of urinary aldosterone

Where cortexone was needed to produce oedema as happened in most of our dogs on a carrot diet one could say that a tendency to

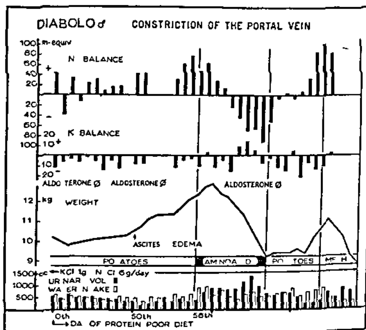

DIABOLO ♂
CONSTRICTION OF THE PORTAL VEIN
m-equiv
N BALANCE
K BALANCE
ALDOSTERONE Ø
WEIGHT
ASCITES
EDEMA
KCl 1g
WATER INTAKE
DAY OF PROTEIN POOR DIET

Fig 6

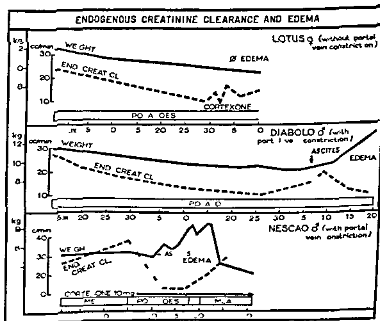

ENDOGENOUS CREATININE CLEARANCE AND EDEMA
LOTUS ♀ (without portal vein constriction)
WEIGHT
Ø EDEMA
END CREAT CL
CORTEXONE
DIABOLO ♂ (with portal vein constriction)
ASCITES
EDEMA
NESCAO ♂ (with portal vein constriction)
EDEMA

Fig 7

SMITH S G and LASATER, T E. (1950) *Proc Soc exp Biol N Y* 74 427
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DISCUSSION

Hokfelt In Sweden there has been one case of primary hyperaldosteronism which was successfully operated upon and which was reported by Dr Skanse and co-workers Prof Wolff made the first assays for aldosterone in the urine of this patient and he was able to show a moderate but significant increase of aldosterone output. We have also assayed this urine and we found an output of 35 µg. per day before operation Six months after the operation we assayed the urine from this patient again and we found that aldosterone was at least not more than 2 µg. per day which is in agreement with what Dr Garrod has just told us I wonder whether these values for aldosterone which remain low several months after operation could be explained on the basis of adrenal atrophy In Dr Skanse s case there was a marked atrophy of the remaining adrenocortical tissue

Garrod I should perhaps have mentioned that the cortisol excretion came back to normal in our two cases whereas the aldosterone excretion stayed very low

Querido Concerning this question of so-called post operative hypoaldosteronism we have to distinguish between two things i e whether we see a low figure or whether we see the clinical state that corresponds to it. We don t yet know all the regulatory factors I wonder whether Dr Garrod s post operative data in this case of 2 µg of aldosterone were actually corresponding to a clinical state of hypoaldosteronism. How did this patient respond to sodium restriction?

Garrod It has not yet been possible to arrange sodium-deprivation tests on these two patients They are feeling so well that they do not want to come into hospital They don t show any clinical evidence of hypoaldosteronism and their plasma electrolyte values are now normal although one of them did go into a sodium losing crisis shortly after operation and had to be given cortexone and extra salt

Querido Did you do a Robinson Kepler water test?

Garrod No

Querido If the water test and sodium deprivation are normal we can then assume that these patients regulatory mechanisms are intact despite the fact that they excrete only 2 µg of aldosterone

Garrod Perhaps so long as they are not subjected to the stress of sodium deprivation

Froesch Concerning the problem of the occurrence of isolated aldosterone deficiency a very interesting observation has been made by Dr Relman and co workers in Boston (to be published*) He has followed a

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NOTE ADDED IN PROOF Since published (1957 *N w Engl J M d*, 257 529).

oedema already existed and was only revealed by cortexone We are inclined to ascribe a similar significance to aldosterone which, however, we do not consider as a constant indispensable determinant factor in oedema formation The very low aldosterone output observed in our dogs also favours this view

These studies of course are related to a particular experimental dog disease ' which in some respects mimics the picture of nephrosis (Metcoff Nakasone and Rame 1954) kwashiorkor (Hansen 1956) cirrhosis and perhaps certain cases of potassium depletion with nutritional defects as reported by Vernet Duckert and Muller (1956) The nutritional and reversible defect appearing in our oedematous animals seems to lie in the cells rather than in the disturbed plasma proteins It is the importance and nature of this defect which seem to determine the response of the animal to cortexone as may be seen from the diuretic effect of amino acids described here

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promote sodium excretion Recently this work has been repeated (Levitt *et al* (1956) *J clin Invest* 25 750) and it has been claimed that injection of calcium salts will impede the tubular reabsorption of sodium But to produce such an effect I believe that hypercalcaemia is needed and I imagine you did not observe this in your subjects

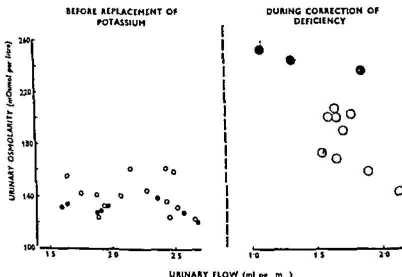


FIG 1 (Stanbury) Defective renal conservation of water in a patient with the "Fanconi syndrome" of adult life

(I C., female aet 35 years. She appeared to have congenital failure of the renal tubular reabsorption of water and had suffered polyuria and polydipsia all her life At aet 35 years she presented with a 300 m-equiv deficiency of potassium)

The open circles refer to the osmolarity of the whole 24-hour specimen of urine numbers within circles refer to successive days of potassium replacement. Black circles refer to short urinary collection-periods after the administration i.m. of 5–10 units of pitressin tannate

Note that while she was potassium deficient there was no renal response to pitressin but the osmolarity of the 24-hour specimens increased from the commencement of potassium replacement On day II when the potassium deficit was completely made up there was a demonstrable response to pitressin although the urinary osmolarity remained below that of the plasma (renal diabetes insipidus) Potassium deficiency appeared to cause complete unresponsiveness to pitressin The defect of water reabsorption remaining after correction of the potassium deficiency was presumably due to her congenital defect The patient's younger sister who had the same type of renal lesion but no potassium deficiency responded in a manner similar to that depicted in the right hand half of the diagram.

(Reproduced from *Advances in Internal Medicine* (1957) Vol 9 by permission of the publishers.)

Morel We have some results complementary to those in this very important communication of Dr Stanbury concerning the possible site of action of mineralocorticoids on the urinary tubule Fig 2 summarizes our present knowledge of the mechanisms of action of these hormones it is agreed that they bring about a tubular reabsorption of sodium by a double mechanism—in part by an exchange with potassium ions and in part, at a more proximal level by an exchange with H+ ions

patient who was admitted to hospital because of Adams-Stokes attacks due to a complete heart block On laboratory examination marked hyperkalaemia and hyponatraemia were noted in the absence of any severe disturbance of renal function Whenever the serum potassium rose above a certain level cardiac arrest occurred and the pacemaker had to be applied several times to revive the patient Resting 17 ketosteroid and 17 hydroxy corticoid levels in the 24-hour urine were normal and the adrenal response to ACTH was well within normal limits. Sodium loading and potassium restriction only partly depressed the serum potassium whereas cortexone and 9-α fluorocortisol in dose levels used for substitution therapy brought the serum potassium back to normal very effectively Aldosterone determined both by means of a bioassay and a physicochemical method, was found to be entirely absent from the urine of this patient It seems as if this patient presents the first well documented case of an isolated hypoaldosteronism with otherwise normal adrenal function

Luft It is very difficult to interpret so-called balance studies if you do not at the same time measure total exchangeable sodium, potassium and chloride intracellular extracellular and total body water I have already mentioned our long term studies with cortexone in normal subjects As you know we have never observed oedema on the contrary we were able to detect a considerable mobilization of sodium (probably inert sodium from the depots in bone) Since cortexone produces polyuria in these patients I wonder whether the additional sodium these patients have to excrete because of this mobilization could account for the polyuria Calcium is also mobilized which indicates that these depots might come from bone Dr Stanbury do you think that this mechanism might be responsible for the polyuria?

Another point is that some years ago long before the aldosterone era we published some cases of longstanding hypokalaemia They were cases of anorexia nervosa they had an isosthenuria low glomerular filtration rate impaired urinary acidification and on autopsy we found minute changes in the tubules of the same type as Dr Stanbury has shown with vacuolization. To our surprise the zona glomerulosa had an increased width and we suggested at that time that this might be related to a salt retaining factor being produced by this enlarged zona glomerulosa (Luft *et al* (1951) *Acta endocr Kbh* 7, 196)

Stanbury I was not certain how much sodium you considered to be mobilized in the course of these experiments but I would suggest that the additional sodium excreted in the urine would be insignificantly small in terms of osmotic load and unlikely to cause polyuria. Even if an additional 1000 m-equiv of sodium were excreted in the course of ten days this should not produce polyuria of the degree observed I think that the polyuria must result primarily from an impairment of water reabsorption. Fig. 1 will show what I mean by a defective renal conservation of water resulting from potassium deficiency The potassium deficiency developing after administration of cortexone and NaCl may produce a similar impaired response to antidiuretic hormone

Luft What would calcium do? You have about 400 mg. of calcium a day excreted by these patients.

Stanbury I should say that about 400 mg. of calcium is totally insignificant, osmotically On the other hand Atchley Loeb and Benedict (1923 *J Amer med Ass* 80, 1643) showed that injected calcium salts could

rats which have received either a water load (white columns) or a salt load (black columns) It can be seen that the osmotic gradient is con siderably reduced after adrenalectomy in rats which had received salt as

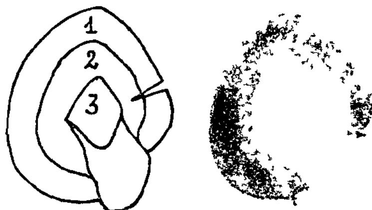


Fig 3 (Morel) Autoradiograph of rabbit kidney slice 3 minutes after i v injection of radioactive potassium At the left scheme of the different regions of the kidney slice (1) cortex (2) medulla (3) papilla.

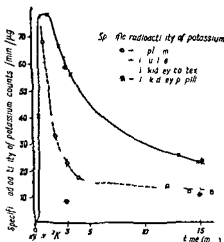


Fig 4 (Morel) Specific radio-activity of potassium in the plasma urine cortex and papilla after an i v injection of radio-active potassium

compared with that of control rats under the same conditions (Guinne bault M and Morel F (1957) C *R Acad Sci* 244 2741) This would seem to indicate that the process of tubular reabsorption of sodium which occurs without Na/K exchange takes place in the ascending limb of

As Dr Stanbury has indicated the potassium of the glomerular filtrate is entirely reabsorbed in the proximal part of the tubule this has been shown by tubular micropuncture and by experiments using radioactive potassium (Morel F (1955) *Helv physiol acta* 13 276) With Mr Guinnebault we have carried out experiments with radioactive potassium on the rabbit which indicate that the potassium of the cells of the kidney cortex is renewed much more rapidly than that of the deeper regions (Fig 3) These experiments show (Fig 4) that the potassium of the cortical cells is the precursor of that of the urine (Morel F and Guinnebault M (1956) *Helv physiol acta* **14,** 255) it thus seems probable that the Na/K

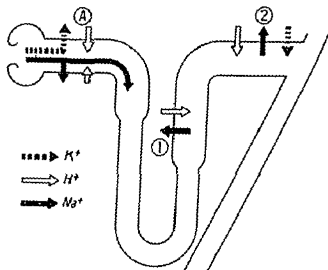


FIG 2 (Morel) Mechanisms of action of mineralocorticoids on urinary tubule.

A reabsorption (nd pend nt of ldo t one) of some of the sodium and all of th potassium filt ed by th gl merulus

1 $Na^+ \rightleftharpoons H^+$ exchange controlled by aldoste one.

2 $N^+ \rightleftharpoons K$ exchange (possibly also $Na^+ \rightleftharpoons H$) cont olled by aldosterone.

exchange controlled by aldosterone occurs at the level of the distal convoluted tubule

The experiments of Wirz have shown that the urine entering the distal convoluted tubule is definitely hypotonic to the plasma and that there exists in the presence of antidiuretic hormone an increasing osmotic pressure of the urine plasma intra and extracellular fluids between the corticomedullary junction and the tip of the papilla The reabsorption of an osmotically active constituent of the urine (viz. sodium) at the level of the ascending limb of Henle s loop could account for these observations We have tried to determine whether the adrenocortical hormones are responsible for this osmotic gradient, by determining the electrolytes at different levels of the kidney (Fig. 5) in control and adrenalectomized

Gross Dr Stanbury can you explain why under treatment with aldosterone potassium loss is definitely less marked than under treatment with cortexone? If the potassium elimination or secretion by the kidney tubules is only a function of sodium reabsorption this difference between the two corticoids is difficult to explain We did some experiments in the rabbit which seems to be the animal most sensitive to potassium loss at least under treatment with cortexone The rabbit develops very rapidly severe muscle paralysis with a potassium level in the plasma which is about 3–3 5 m-equiv /l and in these animals although it is easy to produce muscular paralysis with cortexone we did not succeed in producing muscular paralysis with aldosterone in doses of up to 2 mg per day

One other point in general when one determines plasma potassium one does not know what happens in other tissues for example we find under treatment with cortexone a definite decrease in plasma potassium and a decrease in muscle potassium but in other tissues potassium is not lowered Tobian has already demonstrated that under cortexone the potassium concentration in the aortic wall remains normal or may even increase whereas sodium concentration increases regularly We have confirmed this and have also found that in heart muscle potassium concentration does not diminish in the same way as in skeletal muscle Thus if we determine plasma potassium it has to be kept in mind that we have only an overall picture and do not know how the electrolyte balance in various tissues is influenced by overdosage with either cortexone or aldosterone or other steroid hormones

Stanbury I have no answer to Dr Gross first question his observations are of great interest but I cannot assess their significance His second point concerning the plasma level of potassium and its interpretation is well made Not only does the plasma potassium give you no indication as to how a deficiency of potassium affects the various organs but also a low serum level of potassium is compatible with a perfectly normal bodily content of potassium There is no doubt that the distribution of potassium between the extracellular fluid and the cellular mass taking the latter as an integrated whole can be influenced by changes of pH in body fluids (Scribner *et al*, (1955) *J clin Invest* 34 1276) among other things One also wonders to what extent aldosterone or cortexone can influence directly the distribution of potassium I have made no personal observations on this matter but I believe that in certain cases of hyperaldosteronism the serum level of potassium has remained low until the aldosterone secreting tissue was removed subsequent to operation it has reverted to normal without change in the external balance of potassium I think that is true of Milne s second case but the data have not yet been published fully

Garrod I am intrigued by the differences which Dr Gross finds between aldosterone and cortexone on potassium in his dogs and rabbits Now if it were true that aldosterone was being more rapidly degraded it would be interesting to see what would happen if the aldosterone was given perhaps every six hours or more frequently than every twelve hours Do you think that these differences might then be eliminated?

Gross We tried this we gave it every six hours but also with this sequence of administration we found that the potassium loss in the aldosterone treated animal is definitely less marked than in the cortexone treated one

Henle s loop in precisely that inner region of the kidney where the potassium turnover is slow

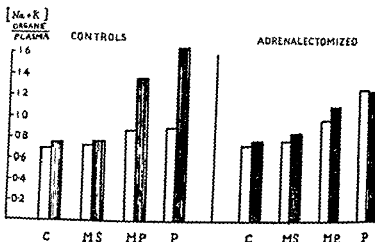


FIG 5 (Morel) Determination of the osmotic gradient between organ and plasma electrolytes (Na and K) at different levels of the kidney in control and adrenalectomized rats which have received either a water load (white columns) or a salt load (black columns) C=cortex MS=external medulla MP=internal medulla P=papilla.

Stanbury I should like to congratulate Dr Morel on his elegant studies His observations on the kinetics of distribution of injected radioactive potassium accord fully with those made by workers in our Department but his extension of it to the actual zones of the kidney is most beautiful All renal physiologists are having to take into account the fine work of Wirz but those of us who are not mathematicians have some difficulty in understanding and applying it. Dr Morel s recent studies should be of the greatest help to us

Gross I should like to congratulate Prof Stahl and his group on the beautiful experiments that he has demonstrated We can only confirm that under high doses of cortexone no oedema will occur in dogs—at least no visible oedema—the only symptom we have found is a slight increase in intravascular volume and a rather low haematocrit value in dogs treated with cortexone There is however an easy way to produce oedema in rats over dosed with cortexone by injecting small doses of renin. This work has already been done by Masson and has been confirmed by Gaunt and collaborators It is very surprising that in rats which have severe kidney damage and which drink per day about the equivalent of their body weight no oedema formation occurs and no retention of water can be detected *Prof Stahl have you observed hypertension in your dogs?* We could not find hypertension in our dogs neither could other workers find regularly an increase of blood pressure in the normal dog under high dosages of cortexone

Stahl We have only occasionally measured the blood pressure in our dogs made polyuric by cortexone and salt no hypertension has so far been observed The blood pressure has not yet been studied in the oedematous dogs

potassium salts Now is it really necessary to give potassium salts to correct the potassium deficiency? In our clinical experience sodium citrate alone is just as effective as sodium and potassium together

Stanbury This is a point which has interested us considerably and we are anxious to make further observations in this syndrome There is a hint in the literature that patients with renal tubular acidosis and potassium deficiency may fall into two groups There are some in whom it has been possible to prevent a subsequent loss of potassium simply by the use of alkaline sodium salts In others although the matter has not been subjected to critical test continued supplementation with potassium salts has appeared to be necessary One suspects that this clinical division of cases may prove to be correct. If one assumes that urinary loss of potassium is in some degree conditioned by secondary hyperaldosteronism it should be possible to prevent such loss by replacing a bodily deficit of sodium and so inhibiting the excessive secretion of aldosterone But you may have a situation analogous to that found in our patients with salt losing nephritis in which a prolonged state of sodium deficiency has led to an apparently autonomous production of aldosterone by the adrenal cortex It is possible that in this circumstance the therapeutic provision of alkaline sodium salts would actually facilitate the urinary loss of potassium and it would then be necessary to provide a supplement of potassium salts (See footnote p 163)

Bartter Following a similar line of reasoning we suggested (1956 *Metabolism* 5, 369) that aldosterone produced in excess as a result of loss of sodium and fluid volume might be responsible for the potassium depletion in renal tubular acidosis It followed that sodium chloride should be as effective as sodium bicarbonate in reducing the urinary potassium loss In one such case that we have studied under a balance regimen however this was clearly not the case Unfortunately we do not have aldosterone values as yet.

Muller Dr Stanbury s digressions on secondary primary hyperaldosteronism were most interesting and in retrospect I wonder whether the case of hypokalaemia which we showed this morning does not have some analogous features Dr Stanbury finds a very high output of aldosterone while his patient is losing 400 m-equiv of sodium whereas the plasma potassium is normal We ourselves found 285 µg of aldosterone at the time when the hypokalaemia had been corrected and the patient was eliminating 200 m-equiv of sodium in her urine However in our case this combination of facts was only transient whereas in Dr Stanbury s case it went on for several months

Stanbury I was greatly interested in Prof Stahl s observations and especially in his having produced simultaneously in the dog both oedema and polydipsia polyuria We have studied a patient whose clinical state was similar to that of Prof Stahl s dogs (Stanbury and Macaulay 1957 *loc cit*) This was a nephrotic child severely depleted of protein who developed an additional deficiency of potassium following treatment with cation-exchange resins Perhaps as a result of his potassium deficiency he then developed additional renal lesions one component of which was hypotonic polyuria. We then had an unusual state of affairs in which an oedematous nephrotic child suffered from severe thirst and excreted more than a litre of urine a day

I should also like to comment on the polydipsia observed by Prof Stahl and by others in animals treated with cortexone and on thirst as a symptom in various forms of potassium deficiency In patients with renal disease we have come to regard the complaint of thirst as an indication that we should look for evidence of potassium deficiency but we are uncertain whether thirst associated with a deficiency of potassium results from an impaired conservation of water by the kidneys or whether it is primarily extrarenal in origin A deficiency of potassium may certainly impair the renal tubular reabsorption of water and affected individuals may have isosthenuria But patients with isosthenuria as a symptom of conventional azotaemic renal failure will rarely excrete more then 2 5 litres of urine daily whereas some patients with hyperaldosteronism who can elaborate isosthenuric urine when stimulated with vasopressin continue to excrete 5 or 6 litres of hypotonic urine daily What compels these patients to produce such large urinary volumes when their kidneys have the potential for cutting it down to 2 5 litres at the most? Is it that potassium deficiency conditions a primary thirst? The observations of Fourman on experimental potassium deficiency in man suggest that this may be a factor We have ourselves observed the disappearance of thirst without accompanying change in the osmolarity of the plasma when a deficit of potassium has been made up and we feel that hypernatraemia which is by no means always present cannot be held responsible for the thirst. Alternatively can hyperaldosteronism influence the symptom of thirst independently of its effect on potassium metabolism? We have had a patient with hyperaldosteronism studied in detail by Wrong, in whom removal of the adrenals produced an immediate and permanent suppression of polyuria and thirst. Previously although this patient could con centrate his urine to 300 m osmol /l he had continued to excrete 3 to 6 litres of hypotonic urine daily

Prader Dr Stanbury I think it is accepted that in renal acidosis there is a sodium and potassium deficiency even if this is sometimes difficult to prove. Usually these patients are treated with a mixture of sodium and

patient was put on a salt free diet, but oedema of the face and ankles persisted In view of the normal kidney functions and cardiovascular system the patient s oedema was considered to be of diencephalic origin Treatment with mercurial diuretics and Diamox was instituted producing each time an increased diuresis on the day of treatment but oliguria on the following days Despite this therapy the oedema persisted and was particularly marked and disfiguring on the face especially in the morning As a result she became depressed and did not continue with her normal activities On July 1st 1956 the patient was admitted to the Clinique théra peutique of the University of Geneva

On admission the patient weighed 67 2 kg and measured 172 cm She presented facial oedema which was particularly marked around the lower eyelids which were dark brown in colour The forehead and cheeks were puffy but showed no pitting upon pressure The hair distribution was normal and of feminine type Neither striae nor obesity were present. The thyroid was of normal size and consistency Several pea sized lymphnodes which had appeared recently were palpable in both axillary regions The liver and spleen were not enlarged

The examination of the heart and lungs was non-contributory and the electrocardiogram was normal Arterial blood pressure was 110/65 Kidney function appeared normal specific gravity of the urine was 1029 there was no albumin sugar or urobilin in the urine Ophthalmoscopic examination showed normal fundi

Haemoglobin was 14 g red-cell count 4 600 000 per mm^3 and white-cell count 11 100 per mm^3 with 69 % polynuclear leukocytes 0 5 % eosinophil 0 5 % basophil 9 % monocytes and 21 % lympho cytes haematocrit 49 %

Non protein nitrogen of the blood was 19 mg /100 ml serum cholesterol was 277 mg /100 ml Total proteins were 5 3 g /100 ml with 2 3 g albumin and 3 0 g globulin Later the total proteins were found to be 5 6 g /100 ml with 2 7 g albumin and 2 9 g globulin Electrophoretic pattern was normal Serum electrolytes were always normal sodium 145 m equiv /l potassium 4 4 m equiv /l calcium 4 6 m-equiv /l chloride between 101 and 107 m equiv /l and carbon dioxide combining power 32 m equiv /l Urinary aldosterone on entry was very high, 107 µg /24 hr Urinary 17 hydroxycorticoids were in the normal range between 7 and 9 mg / 24 hr (method of Reddy)

IDIOPATHIC OEDEMA WITH HYPERALDOSTERONURIA

René S Mach

Clinique thérapeutique universitaire Geneva

FOR many years we have been interested in the unusual clinical picture observed among those patients who present chronic generalized oedema without any past or present history of renal cardiac or hepatic disorders Oedema is the consequence of excessive sodium retention and varies strictly with the salt content of the diet The condition does not grow worse over a period of years nor does it become complicated by arterial hypertension or by disturbances of potassium metabolism Such a syndrome has been described in the literature under the term isolierte Kochsalzstoffwechselstorung by Jungmann (1922) and oligurie durch Salzretention by Lauda and Wichtel (1936) Since the underlying disorder is not known the condition has also been called idiopathic oedema

In 1955 we published the case of a patient presenting this particular type of oedema as well as an increased aldosterone excretion (Mach *et al* 1955) It appeared plausible to us that a relationship might be established between the retention of salt and water and the hyperaldosteronuria In 1956 Thorn and co workers published a similar case of a patient who had presented over a period of twenty years a syndrome of NaCl retention with oedema which upon further investigation was found to be accompanied by hyperaldosteronuria The oedema disappeared after removal of one adrenal gland which contained an adenoma (Thorn *et al* 1957) but it reappeared a few months later and removal of the second adrenal became necessary Luetscher and Lieberman (1957) reported several similar cases under the heading Idiopathic Edema with increased aldosterone output An additional case of this syndrome is presented here

Case A G a 37 year old woman had always been in excellent health and had never had any sign of renal or cardiac disorder However in August 1955 several weeks after a great emotional upset she noticed that her eyelids were beginning to swell and that she had gained 5 kg Each time she increased her NaCl intake an aggravation of the oedema resulted which now became generalized The

mercurial diuretic which was then administered, resulting immediately in a substantial diuresis

Fig 1 shows the effect of these large doses of NaCl increase in weight and decrease in diuresis The poor excretion of NaCl which was practically insignificant (2 5 m equiv) on the first day rose to only 35 m equiv on the third day Thus of the entire 510 m equiv of sodium received by the patient during the three days only 54 m equiv were eliminated i e 90 % was retained The previous very high values of urinary aldosterone fell to 35 20 and 22 µg /24 hr These values however are much too high to allow a normal elimination of NaCl

A second similar large dose of NaCl shows the same degree of NaCl retention and an equally insufficient lowering of the aldosterone (Fig 1) In the course of these two tests we did not observe any change in the potassium excretion and the urinary 17 hydroxycorticoids determined according to the method of Reddy remained constant

FOLLOW UP STUDIES

Despite a very severe restriction of salt intake the oedema persisted In order to diminish the secretion of aldosterone a small increase in the salt intake was allowed However the patient was unable to tolerate it In a further attempt to lower the aldosterone values we asked the patient to increase her fluid intake considerably The response to this treatment was favourable (Fig 2) and the aldosterone excretion dropped as low as 15 to 20 µg /24 hr despite sodium restriction Thus lowering of the aldosterone improved the elimination of salt and water In order to prevent the usual rebound increased aldosterone secretion which follows the administration of mercurial diuretics we asked the patient to avoid them in the future Under these circumstances i e increased fluid intake with salt restriction the patient remained relatively well and was able to resume her normal activities despite the persistence of some early morning facial oedema

DISCUSSION

The generalized oedema which this patient has presented over a period of two years has always been closely related to the sodium content of her diet The oedema cannot be explained on a cardiovascular or renal basis The hepatic function tests were also normal

EFFECT OF LARGE DOSE OF NaCl

To the strict salt free diet which the patient had been following a supplement of 5 g of NaCl was added on the first day 10 g on the

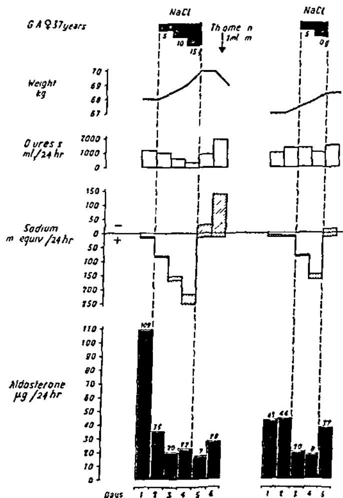


FIG 1 Two salt loads at 2 months interval in a 37 year-old patient with idiopathic oedema. Note considerable retention of sodium chloride because of inadequate lowering of the urinary aldosterone levels

second and 15 g on the third day The facial oedema increased to an extreme degree orthopnoea developed and the patient complained of headaches and a feeling of anxiety She insisted upon having a

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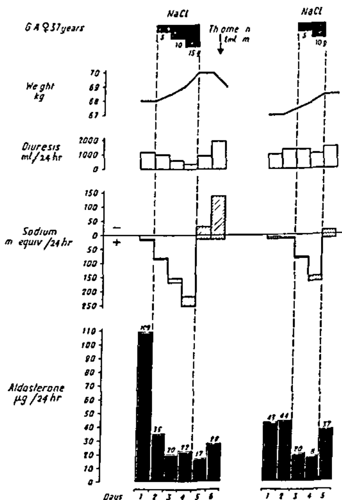


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of aldosterone—whether spontaneous or as a result of therapy—the retention of sodium was less marked but we have never observed a period with low enough aldosterone values to allow the complete disappearance of the oedema Only a complete remission of the sodium retention syndrome with the normalization of the urinary aldosterone values would give us the final proof of a cause and effect relationship

Our patient responded to the increased level of aldosterone in a classic manner as did our first case reported in 1955 and the one presented by Thorn i e by extracellular retention of salt and water with resulting oedema Thus the syndrome may be distinguished from the one described by Conn (1955) in which there was intra cellular retention of sodium without oedema loss of potassium polyuria and arterial hypertension The syndrome of hyperaldosteronuria with accompanying oedema is not a spectacular one and many such cases may even pass unnoticed or be confused with some other disorder The prognosis is good the condition may be improved by very simple dietary measures and surgical intervention is unnecessary

What is the cause of the hyperaldosteronuria presented by these patients? In Thorn s patient who had oedema of unknown origin over a period of twenty years an adenoma of one adrenal gland was found Removal of the gland containing the tumour resulted in temporary disappearance of the oedema as well as in decreased aldosterone excretion which subsequently became normal For a few months following the operation the patient was able to eliminate a sodium load normally but later the aldosterone secretion increased again and sodium retention resulted necessitating the removal of the other adrenal gland (Crabbe *et al* 1957)

Since our own two patients did not undergo exploratory surgery we cannot give a final verdict on the appearance of their adrenal glands But if we were to judge from the chronic evolution of their disorder with its periods of partial remission we would be inclined to suspect that a disturbance in the regulation of aldosterone secre tion is at fault and not an adrenal tumour or bilateral adrenal hyperplasia It is therefore most likely that some homeostatic mechanism is disturbed so that the aldosterone secretion is no longer correctly regulated by normal stimuli

Certain observations indicate the participation of the central nervous system in this disturbance We find among these patients

and the slight hypoproteinemia which disappeared later, cannot account for the oedema nor could we attribute the patient s oedema to local circulatory factors or permeability changes

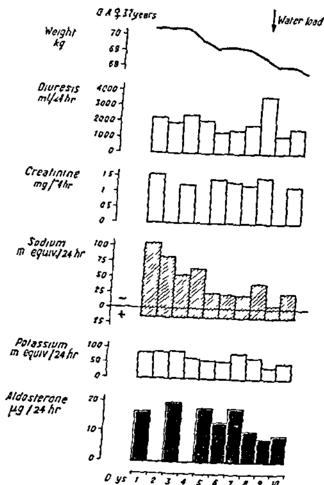


FIG 2 Water load in a 37 year old patient with idiopathic oedema. Note the slight, but significant, lowering of the urinary aldosterone levels

Thus we are inclined to establish a cause and effect relationship between the hyperaldosteronuria and the persistent oedema The increased aldosterone secretion with its consequent effects on sodium and water metabolism could therefore be considered as the basis of the whole clinical syndrome Whenever there was a diminution

ALDOSTERONURIA IN OEDEMA

H P Wolff K. R Koczorek and E Buchborn
First Medical Clinic of the University of Munich

The study presented here was designed to furnish data on aldosterone activity in hydropic disease of cardiac and hepatic origin and to define the role of hyperaldosteronism in the production of oedema and ascites

Aldosterone was determined according to the physicochemical method of Neher and Wettstein (1956) Urinary aldosterone and urinary sodium were determined in 55 patients with hydropic and non hydropic liver disease The results of this study are shown in Fig 1 Twenty healthy subjects with normal salt intake served as controls for patients with acute hepatitis Ten normal subjects under moderate sodium restriction (60–80 m-equiv per day) served as controls for the patients with liver cirrhosis who were submitted to the same diet In order to outline more clearly the pattern of aldosterone activity in liver cirrhosis and to avoid interfering effects due to sodium deprivation these cases were not submitted to severe sodium restriction Out of 12 cases of acute hepatitis urinary aldosterone was raised in some cases and in others was normal while urinary sodium was decreased in some cases and in others was normal A similar distribution pattern of aldosterone and sodium excretion was observed in 12 patients with compensated liver cirrhosis In these two groups an increase in urinary aldosterone was generally found when loads of salt and water were excreted incompletely or with considerable delay and when clinical and biochemical findings indicated advanced impairment of liver function

Chart and co workers (1956) have shown that aldosterone is inactivated *in vitro* by liver tissue Impairment of liver function may therefore alter the normal equilibrium between aldosterone secretion breakdown and excretion and may result in abnormal aldosterone activity in blood and urine In order to obtain preliminary data on aldosterone metabolism in liver disease we loaded normal subjects and patients having compensated liver cirrhosis with 500 μg aldo sterone acetate and compared their aldosterone excretions In the normal subjects urinary aldosterone was raised during 30 hours

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certain significant characteristics in their behaviour very often the oedema is influenced by emotional factors The patient reported here first noticed the oedema a few weeks after the death of her husband and it seems to have somewhat diminished now that she has accepted her new situation It is difficult to estimate the exact rôle played by the central nervous system in the regulation of the endocrine glands but it is interesting to mention in this connexion the experiments of Farrell and co workers (1957) who were able to produce a discharge of aldosterone measured in the adrenal veins of the dog after the injection of extracts prepared from the hypothalamus

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[Discussion of this paper was postponed until after the paper by Wolff and co-workers.—EDS]

accumulation of ascites, 2–14 days following discharge of ascites by abdominal paracentesis In these cases the striking increase in aldosterone activity cannot be explained satisfactorily on the basis of impaired steroid inactivation alone It may be assumed that in the production of ascites internal shifts of sodium and water play a decisive role in the activation of adrenocortical aldosterone secretion To evaluate these factors simultaneous studies on plasma volume

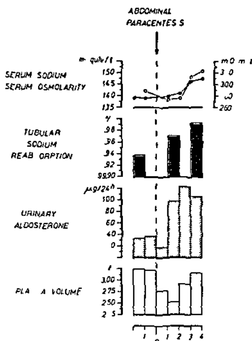


Fig 2 Effect of abdominal paracentesis (9 5 1) on plasma volume urinary aldosterone tubular sodium reabsorption serum sodium and effective serum osmolarity in a patient with decompensated liver cirrhosis.

urinary aldosterone renal function sodium metabolism and extra cellular tonicity were carried out in 8 patients following abdominal paracentesis (Wolff Koczorek and Buchborn 1957*a* Wolff *et al* 1956*a*)

The experimental data shown in Fig 2 suggest that the following sequence of events results in hyperaldosteronism and renal retention of sodium

(1) Intravascular loss of sodium and water by excessive tran sudation of fluid into the peritoneal cavity This loss is indicated by a transitory decrease in plasma volume

following injection and 4–6 % of the aldosterone load was recovered in the urine In the patients with cirrhosis aldosterone excretion was raised during 60 hours following injection, and approximately 15 % of the aldosterone load was recovered These findings suggest impaired aldosterone metabolism in liver

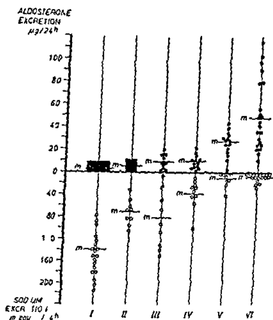


FIG 1 Urinary aldosterone and urinary sodium in normal persons and patients with liver disease

I normal persons with free salt intak
II normal p rsons under moderate sodium restri tion (60–80 m equ /day)
III pati nts w th acute hep t t s
IV patient with compensated liver cirrho is
V p t ent w th decompensated live cirrhosis before ab d minal p racentesis
VI patients w th decompen ted li er cirrhosis 2–14 days following abdominal paracentesis.

disease However our experiments are too limited in number and the experimental technique used seems too rough to allow final con clusions to be drawn

In decompensated liver cirrhosis giving rise to ascites high urinary aldosterone and low urinary sodium were observed regularly These findings are in accordance with those of Chart and Shipley (1953) and Axelrad and co workers (1955) The highest aldosterone activities and strongest sodium retention were found in acute

correlation between serum osmolarity and plasma adiuretin This correlation proved valid in 30 normal individuals as well as in more than 40 patients with acute hepatitis compensated and decompensated liver cirrhosis and heart failure

Exceptions to this rule were found only when discharge of ascites was followed by acute and severe changes in plasma volume In

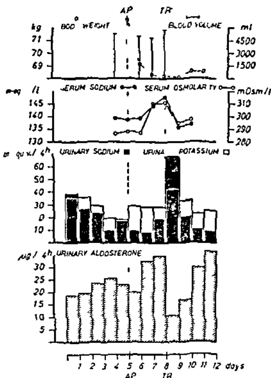


FIG 3 Effect of abdominal paracentesis (4 8 l) and blood transfusion (1000 ml) on blood volume urinary aldosterone urinary sodium urinary potassium, serum sodium and effective serum osmolarity in a patient with decompensated liver cirrhosis

these cases decrease in plasma volume was followed by high plasma adiuretin despite low serum osmolarity and increase in plasma volume due to reflux of gross peripheral oedema was accompanied by low plasma adiuretin despite high serum osmolarity These observations are consistent with the findings of Leaf and Mamby (1952)

In summary of these observations on acute production of ascites loss of intravascular sodium and water leads to an increase in aldosterone secretion tubular sodium reabsorption and body sodium The resulting changes in the ratio of extracellular to intracellular

(2) Compensatory shift of intracellular sodium and water to the extracellular compartment This shift is indicated by a transitory decrease of effective serum osmolarity haematocrit and total plasma protein despite limited water intake At the same time the sodium and water content of the erythrocytes decreases These findings may be regarded as indications of secondary changes in intracellular hydration and electrolyte pattern which may effect by ways still unknown, adrenocortical aldosterone production

(3) Increase in adrenocortical aldosterone secretion indicated by increase in urinary aldosterone

(4) Renal retention of sodium demonstrated by increase in tubular sodium reabsorption and decrease in urinary sodium

(5) Increase in body sodium and extracellular tonicity This increase is indicated by an increase in the sodium and water content of the red cells (Riecker Koczorek and Wolff 1957) and by a rise in effective serum osmolarity following an initial decrease

These changes lead by ways discussed later to stimulation of posterior pituitary antidiuretic acitivity and renal retention of water

When the intravascular fluid of these patients was augmented by a transfusion of 1000 ml blood on the third or fourth day following abdominal paracentesis the raised aldosterone activity responded with a strong transitory decrease followed by renal release of sodium and decrease in serum osmolarity (Fig 3) These observations show that the mechanism outlined above can be reversed for a limited period by a transitory replacement of the intravascular fluid loss

The data presented so far furnish information only on the mechanism leading to retention of sodium To define the connecting links between hyperaldosteronism and renal retention of water in hydropic liver disease we extended our studies by simultaneous examination of tubular water reabsorption and by estimation of plasma antidiuretic activity using a new and we think specific technique for the quantitative determination of adiuretin (ADH) described by Buchborn (1955)

As we have shown before increase in aldosterone activity leads to increase in body sodium and extracellular tonicity The work of Verney (1948) has already demonstrated that the antidiuretic mechanism of the neurohypophysis is sensitive to changes in effective solute concentration of the extracellular fluid Our analytical data establish a constant ratio between changes in extracellular tonicity and posterior pituitary antidiuretic activity expressed in a positive

lost into the peritoneal cavity and to maintain a sufficient circulating volume In normal individuals however an increase in body sodium and water reduces aldosterone activity to low levels In patients in whom *ascites is being produced* retention of sodium and water fails to reduce aldosterone activity This failure might be explained as has been done by Duncan *and co-workers (1956) by the fact that* the retained fluid shifts to the peritoneal cavity and is segregated in a body compartment which is not sensitive to changes in its content of sodium and water This failure to reduce aldosterone activity following fluid retention plays an important role in the ever increasing accumulation of ascites present in many patients with decompensated liver cirrhosis

We have studied aldosteronuria in hydropic heart disease in 48 patients with different forms and in different stages of decompensated heart failure (Wolff Koczorek and Buchborn 1957*b* Wolff *et al* 1956*b*) The results of this study are shown in Fig 5 Twenty healthy subjects with normal salt *intake served as controls for the* heart patients classified as untreated Ten normal subjects on moderate salt restriction (60–80 m equiv sodium per day) served as controls for the heart patients submitted to the same diet during therapy Since hyperaldosteronism in hydropic heart disease is induced primarily by pathological changes in cardiovascular haemodynamics these subjects were submitted to only moderate salt *restriction in* order to outline more clearly the adrenocortical response to circulatory improvement attained by digitalis and bedrest, and to avoid interfering effects *due to sodium depletion*

The aldosterone and sodium excretion by 18 untreated patients with severe peripheral oedema ascites and pulmonary congestion showed no uniform pattern Urinary aldosterone was increased and urinary sodium was diminished in all patients who retained water and gained weight as symptoms of advancing cardiac decompensation These patients represented the majority of the cases under study (Fig 5) *In a minority urinary aldosterone* and urinary sodium were within the normal range When put on a diet containing 60–80 m equiv sodium per day these patients retained little or no salt and water These cases apparently represented the situation where a transitory equilibrium had been established between the reduced myocardial capacity and adjusting changes in circulation distribution of salt and water and in the patients way of life

Under treatment with digitalis moderate sodium restriction and

tonicity and/or the intravascular volume defect stimulate neurohypophyseal secretion of adiuretin and tubular reabsorption of water (Fig 4)

These observations seem to furnish experimental proof for the suggestion of Bartter and co workers (1956) that the homeostatic regulation of body sodium and water depends on a dual feedback mechanism where aldosterone controls body sodium and adiuretin

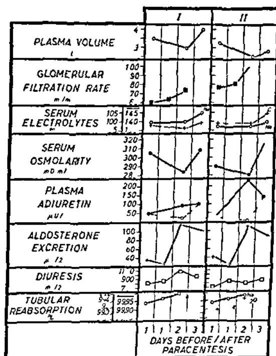


Fig 4 Hormonal control of body sodium and water during acute formation of ascites following abdominal paracentesis in 2 patients with decompensated liver cirrhosis

controls body water The response pattern of this mechanism was found to be qualitatively the same in normal individuals and in patients with hydropic disease of various origin (Wolff Koczorek and Buchborn 1957 *a* and *c*)

These studies of acute production of ascites following abdominal paracentesis may also serve as a model outlining the retention mechanism in spontaneous production of ascites when patients with liver cirrhosis enter the stage of decompensation (Schwiegk 1955) They suggest that an increase in circulating aldosterone followed by retention of sodium and water tends to replace the sodium and water

lost into the peritoneal cavity and to maintain a sufficient circulating volume In normal individuals however an increase in body sodium and water reduces aldosterone activity to low levels In patients in whom ascites is being produced retention of sodium and water fails to reduce aldosterone activity This failure might be explained as has been done by Duncan and co workers (1956) by the fact that the retained fluid shifts to the peritoneal cavity and is segregated in a body compartment which is not sensitive to changes in its content of sodium and water This failure to reduce aldosterone activity following fluid retention plays an important rôle in the ever increasing accumulation of ascites present in many patients with decompensated liver cirrhosis

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The aldosterone and sodium excretion by 18 untreated patients with severe peripheral oedema ascites and pulmonary congestion showed no uniform pattern Urinary aldosterone was increased and urinary sodium was diminished in all patients who retained water and gained weight as symptoms of advancing cardiac decompensation These patients represented the majority of the cases under study (Fig 5) In a minority urinary aldosterone and urinary sodium were within the normal range When put on a diet containing 60–80 m equiv sodium per day these patients retained little or no salt and water These cases apparently represented the situation where a transitory equilibrium had been established between the reduced myocardial capacity and adjusting changes in circulation distribution of salt and water and in the patients way of life

Under treatment with digitalis moderate sodium restriction and

tonicity and/or the intravascular volume defect stimulate neurohypophyseal secretion of adiuretin and tubular reabsorption of water (Fig 4)

These observations seem to furnish experimental proof for the suggestion of Bartter and co workers (1956) that the homeostatic regulation of body sodium and water depends on a dual feedback mechanism where aldosterone controls body sodium and adiuretin

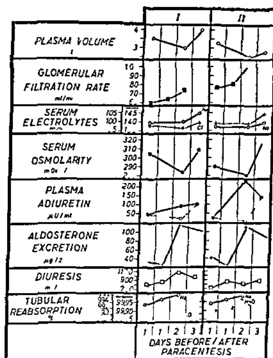


FIG 4 Hormonal control of body sodium and water during acute formation of ascites following abdominal paracentesis in 2 patients with decompensated liver cirrhosis

controls body water The response pattern of this mechanism was found to be qualitatively the same in normal individuals and in patients with hydropic disease of various origin (Wolff Koczorek and Buchborn 1957 *a* and *c*)

These studies of acute production of ascites following abdominal paracentesis may also serve as a model outlining the retention mechanism in spontaneous production of ascites when patients with liver cirrhosis enter the stage of decompensation (Schwiegk 1955) They suggest that an increase in circulating aldosterone followed by retention *of sodium and water tends to replace the sodium and water*

lost into the peritoneal cavity and to maintain a sufficient circulating volume In normal individuals however an increase in body sodium and water reduces aldosterone activity to low levels In patients in whom ascites is being produced retention of sodium and water fails to reduce aldosterone activity This failure might be explained as has been done by Duncan and co workers (1956) by the fact that the retained fluid shifts to the peritoneal cavity and is segregated in a body compartment which is not sensitive to changes in its content of sodium and water This failure to reduce aldosterone activity following fluid retention plays an important role in the ever increasing accumulation of ascites present in many patients with decompensated liver cirrhosis

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Under treatment with digitalis moderate sodium restriction and

diuretics the aldosterone and sodium excretion of 20 hydropic heart patients showed considerable variations During improvement of myocardial function and mobilization of oedema, however

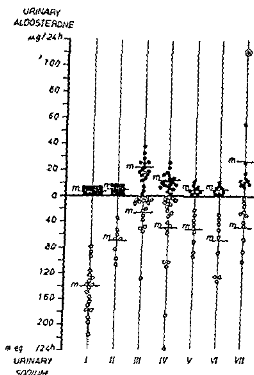


Fig 5 Urinary aldosterone and urinary sodium in decompensated heart failure

I normal persons with free salt intake
II normal persons under moderate sodium restriction (60–80 m-equiv /day)
III patients with hydropic left + rightsided failure untreated
IV patients with hydropic left + rightsided failure during treatment with digitalis bed rest and moderate sodium restriction (60–80 m-equiv /day)
V patients with hydropic left + rightsided failure recompensated after treatment
VI *patients with leftsided heart failure*
VII patients with rightsided heart failure
⊕ = cardiac cirrhosis

urinary aldosterone decreased and urinary sodium increased gradually Following *complete recompensation* aldosterone and sodium excretion was generally within the normal range

When digitalis treatment of hydropic heart failure was supplemented by severe sodium restriction by means of resins (30–60 g /day)

urinary aldosterone responded in two different ways (Wolff Koczorek and Buchborn 1957*b*)

In 3 patients with slow mobilization of oedema aldosterone excretion increased considerably during intake of resins (Fig 6 A) In one patient with rapid and excessive mobilization of oedema

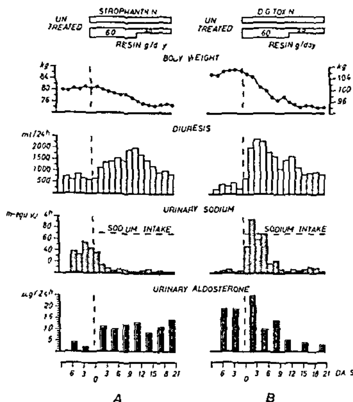


Fig 6 Different responses of untreated patients with hydropic left + rightsided failure to heart therapy supplemented by cation exchange resins

aldosterone excretion slowly decreased to normal (Fig 6 B) These different adrenocortical responses to severe sodium restriction may be explained on the basis of a different response of myocardial fluid dynamics to digitalis and sodium restriction Rapid improvement of myocardial function with instant mobilization of oedema leads to an excessive transfer of sodium and water from the interstitial space and the body cavities into the intravascular compartment

This transfer may be compared to an infusion of saline solution in normal persons and must meet with an adequate response i e reduction of aldosterone activity and renal release of sodium In patients with slow myocardial response and slow mobilization of hydrops the salt and water depots represented by ascites and oedema are not available to compensate the effects of sodium loss Here sodium restriction must meet the opposite response i e increase in urinary aldosterone and renal retention of sodium

In 9 patients with leftsided heart failure exhibiting pulmonary congestion of varying degree but no symptoms of systemic congestion urinary aldosterone and urinary sodium were generally within the normal range (Fig 5) In 11 patients with rightsided heart failure exhibiting systemic congestion but no symptoms of pulmonary congestion the results were not uniform (Fig 5) Eight cases with advanced peripheral oedema and ascites showed sodium retention and a moderate to excessive rise in aldosterone activity The remaining 3 presented normal excretion of aldosterone and sodium They were free of ascites and gross oedema at the time of study These findings are consistent with the clinical observation that marked sodium retention occurs mainly in patients with systemic congestion and high venous pressure whereas patients with pulmonary congestion but normal venous pressure are generally capable of excreting salt and water loads

It can be assumed that hyperaldosteronism in heart failure is primarily induced by pathological changes in cardiovascular haemodynamics which lead to abnormal distribution of blood water and electrolytes To define the response of the aldosterone mechanism to cardiovascular decompensation or recompensation it is essential to study urinary aldosterone and sodium metabolism under conditions which provide significant changes in myocardial function and prevent interfering effects due to overload or deprivation of salt and water

When severe decompensation was produced by means of physical stress in a heart patient with constant normal intake of sodium (100 m-equiv /day) and water (1000 ml /day) there was an increase in body weight venous pressure and urinary aldosterone while diuresis and urinary sodium fell considerably (Fig 7) When complete recompensation was achieved in the same patient on the same diet by bedrest and digitalization only body weight venous pressure urinary aldosterone diuresis and urinary sodium returned to normal

Phlebotomy of 500 ml blood at the climax of decompensation effected a response which was qualitatively the opposite of that which we have seen in normal individuals According to Starling s law the cardiovascular haemodynamics of this highly decompensated heart patient must have been operating in the descending limb of Starling s curve Thus decrease in venous pressure due to venepuncture apparently induced a transitory improvement in myocardial function followed by a transitory improvement in the pathological distribution of blood water and electrolytes

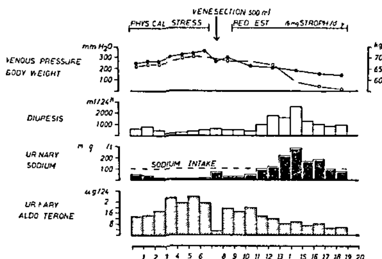


FIG 7 Response of urinary aldosterone and urinary sodium to decompensation evoked by physical stress venesection (500 ml) and to recompensation achieved by bedrest and strophanthin in a patient with decompensated left + rightsided heart failure under constant sodium intake (100 m equiv /day)

In seeking a significant relationship between aldosterone activity and certain changes in cardiovascular haemodynamics we submitted 10 heart patients to heart catheterization and determination of stroke volume and cardiac output No strict correlation was found between urinary aldosterone and right ventricular end diastolic pressure but urinary aldosterone was generally increased in patients with high central venous pressure No strict correlation was found between urinary aldosterone and cardiac output but most patients with decreased cardiac output showed urinary hyperaldosteronism Increase in venous pressure leads to loss of intravascular sodium and water into the interstitial space and into the

body cavities and decrease in cardiac output leads to low arterial blood volume Probably both factors—loss of sodium and water on the venous side and reduced plasma flow on the arterial side—contribute to a pathological distribution of blood electrolytes and water, resulting in activation of the aldosterone mechanism

In conclusion, some observation on aldosterone excretion in toxaemia of pregnancy may be mentioned (Fig 8) As Venning and Dyrenfurth (1956) have shown and as we have found also urinary aldosterone rises in the course of pregnancy to very high levels and returns to normal immediately after delivery The cause of the striking increase in aldosterone activity is so far unknown but changes in the distribution of water and electrolytes or impaired aldosterone metabolism in pregnancy must be considered The latter assumption is strengthened by the observation that blood levels and excretion of cortisol cortisone and progesterone are also greatly increased during pregnancy (Robinson *et al* 1955 Koczorek and Wolff, 1957) These steroids are known to promote sodium excretion under certain conditions (Landau *et al* 1954 Gross 1956) Thus normal sodium balance in pregnancy may be maintained by equilibrium of sodium diuretic and sodium retaining steroids at a higher level of activity In 18 patients with toxaemia of pregnancy aldosterone excretion unexpectedly did not exceed the values found in normal pregnant females On the contrary several cases of severe eclampsia showed lower excretion of aldosterone than did normal females in the same stage of pregnancy It has been shown (Venning Singer and Simpson 1954) that in severe toxaemia excretion of cortisol and progesterone is considerably reduced It seems possible that in these cases an excess of aldosterone may result leading to retention of sodium and contributing to the production of toxaemic oedema

When aldosterone excretion of 249 subjects from the different groups studied was plotted against sodium excretion several different correlations resulted (Fig 9) (Buchborn Koczorek and Wolff 1957)

In summary maximal renal sodium retention in hydropic heart failure was accompanied by lower aldosterone activity and maximal sodium retention in hydropic liver cirrhosis was accompanied by higher aldosterone activity than was observed in normal individuals submitted to severe sodium restriction In normal pregnancy and in some cases of acute hepatitis high aldosterone activities were accompanied by normal sodium output Analysis of renal function in patients from the different groups showed that sodium output was

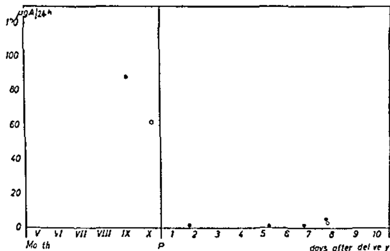


FIG 8 Urinary aldosterone in normal (●) and in toxaemic (○) pregnancy

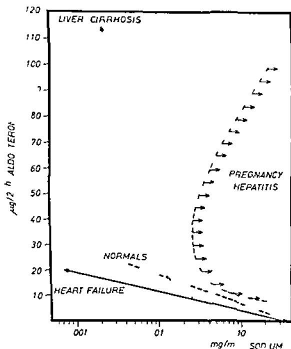


FIG 9 Ratio of urinary aldosterone to urinary sodium in hydropic heart failure decompensated liver cirrhosis acute hepatitis and normal pregnancy

dependent on tubular sodium reabsorption whereas changes in glomerular filtration rate had little additional influence Thus the different ratios of urinary aldosterone to urinary sodium must be due to other factors Among these must be considered impaired hepatic aldosterone metabolism changes in the tubular response to aldo sterone induced by changes in acid base equilibrium and by the presence of other steroids promoting sodium excretion These findings can only be considered as a starting point for more extensive studies

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DISCUSSION

Bartter I would like to congratulate Prof Wolff on the elegant studies he has presented

As regards the effect of sodium depletion on cardiac patients our experience is quite similar to his This may be illustrated from two studies (Duncan, L. E. Jr Liddle G W and Bartter F C (1956) *J clin Invest* 35 1299) In the majority of the patients as one removes sodium aldosterone excretion goes up exactly as in the normal Fig. 1 shows a study

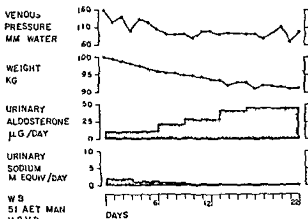


FIG 1 (Bartter) Effect of cation exchange resin and sodium restriction on venous pressure body weight and urinary aldosterone and sodium in a 51 year-old man with congestive heart failure.

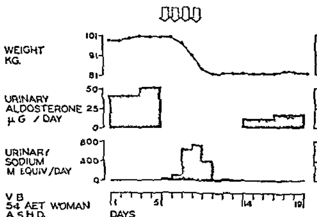


FIG 2 (Bartter) Effect of cation exchange resin sodium restriction and mercurial diuretic on body weight and urinary sodium and aldosterone in a 54-year-old woman with congestive heart failure

in which sodium was removed with exchange resin Aldosterone excretion rose to high values In some patients however the response to sodium deprivation is quite the opposite Fig 2 shows a study wherein sodium was removed with mercurial diuretics and an exchange resin Aldosterone excretion fell Thus only the latter exceptional cardiac was retaining sodium less avidly after he was improved by therapy

Prof Wolff have you any explanation for the fall of serum osmolarity which you found after the removal of ascites? Since the patient s total water intake was restricted and there was no weight gain must not the intracellular osmolarity be increasing as that of serum decreases? How do you explain this apparent movement of water against an osmotic gradient?

Wolff Perhaps the following observations offer some explanation In 8 out of 12 cases studied aldosterone activity showed a short transitory decrease on the first day following abdominal paracentesis We do not know the exact reason but we think that the instant reflux of peripheral oedema after ascites discharge may act like an infusion of saline and reduce aldosterone activity for a limited period Decrease of aldosterone activity leads to renal release of sodium resulting in a decrease of extracellular tonicity as expressed in a fall of serum osmolarity Dr Riecker in our group found that at the same time water and sodium move from the erythrocytes into the intravascular space If the red cells are representative in this respect for the intracellular compartment it can be concluded that the cellular water did not move across the osmotic gradient On the second day after abdominal paracentesis when excessive transudation of intravascular fluid into the peritoneal cavity set in aldosterone activity tubular reabsorption of sodium and serum osmolarity rose At the same time sodium and water were found to enter the red cells It is Dr Riecker s wish that these data on aldosterone activity and the sodium and water content of the erythrocytes should be accepted as preliminary their number being too limited to permit final conclusions to be drawn from them

Bartter How was the red cell sodium and water determined?

Wolff Isolated red cells were dried until their weight remained constant and the water content was calculated from the decrease in weight The sodium content of the isolated erythrocytes was determined directly by flame photometry

Bartter You said that your patients with right sided failure had high urinary aldosterone values in general and your patients with left sided failures did not which I consider to be an important observation Then did you not say that there was no correlation of aldosterone output with right ventricular end diastolic pressure?

Wolff Not a strict correlation in the mathematical sense but generally patients with increased central venous pressure exhibited increased aldosterone

Baulieu Prof Wolff s observations in pregnancy agree very well with our observations on cortisol which demonstrate that there is not an increased production operating in pregnancy but an impaired metabolism The liver is incapable of an adequate hydrogenation and conjugation of the free hormone This is also observed in hepatitis as Prof Wolff has shown for aldosterone

Desaulles It is surely very difficult to know exactly what happens during pregnancy It has been proved that there is a very much higher production of progesterone or progesterone like compounds whether or

not there is an augmented production of genuine cortisol like compounds is still a matter of controversy This can be measured indirectly taking advantage of the anti inflammatory effect of cortisol and cortisol like compounds Meyer and co-workers (Meyer R K. Stucki J C and Auslebrook K. A (1953) *Proc Soc exp Biol N Y* 84 624) showed during the second stage of gestation in rats a clearcut and very marked anti inflammatory reaction and traced it to the placenta only (Stucki J C. and Meyer R K. (1955) *Endocrinology* 57, 173) The presence of the functional placenta is solely responsible for this anti inflammatory reaction We have extended the work not only to rats but to different animals guinea pigs and rabbits and this type of reaction is always found in the second half of gestation So it seems to me that during this period there must be a high content of anti inflammatory substances in the blood

Baulieu Klotz and I have seen a patient whose case report is interesting in connexion with the findings of Prof Mach and Dr Muller This was a neurotic female slightly obese and her oedema and sodium retention were present only when she was in an upright position, and disappeared when she was in bed In a parallel manner with the same sodium intake the aldosterone excretion was definitely high when she was standing and went down to normal when she was in the lying position

Basteme In connexion with the case described by Prof Mach I would like to comment briefly on a case of idiopathic oedema which was not due to aldosterone. This patient is now 25 years old Since the age of 7 she complained of troublesome swelling of the legs At the age of 18 the oedema spread to the arms and the face and it became very troublesome the following year during pregnancy Soon after delivery we had the opportunity to carry out some studies and we could find no disturbances in cardiac renal hepatic or thyroid functions which might explain this oedema Her blood proteins were normal 6 3 g./100 ml. with a normal albumin/globulin ratio the extracellular compartment was markedly increased (from 24 to 29 per cent) Water loads were well eliminated There was a certain degree of acrocyanosis which could not be corrected by periarterial novocaine infiltrations The patient was admitted again in September 1956 and in May 1957 She still complained of the same symptoms

The changes in the rate of water diuresis sodium output urinary anti diuretic hormone and 17 ketosteroid output have been studied during a period of restricted NaCl intake and on a 15–30 g salt intake Subsequently a similar sodium load was given while the patient was on 100 mg. of cortisone acetate per day During the first period of salt restriction the weight and the sodium output decreased the antidiuretic hormone output remained high higher than we found in normal people After a large load of salt the weight increased there was a slight sodium diuresis but there was a very marked rise in the antidiuretic hormone and later also a rise in 17 ketosteroid output Chromatographic separations of the various fractions according to the method of Dingemanse have shown that from the beginning there was a marked increase in fractions 6 and 7 which corresponds to oxygenated C_{21} compounds Following a second period on a salt free diet we repeated a second sodium load this time with 100 mg. cortisone per day under these conditions there was no antidiuretic response and no output of 17 ketosteroid

Later we repeated the same experiment with salt intake first without any other treatment and then with amphenone (3 g per day) During the first

experiment we again found a marked increase of antidiuretic hormone and the patient retained salt and water When amphenone was given there was no sodium diuresis

We think we have evidence that this patient s oedema was not due to a hypersecretion of aldosterone Firstly the aldosterone level was normal on a salt free diet secondly under amphenone treatment there was no sodium diuresis and thirdly the Na/K ratio in the urine never fell below 0 5 However throughout the experiment we found a very high output of antidiuretic hormone in the urine

Mach Prof Bastenie s case is very interesting and it illustrates exceedingly well the complexity of the clinical picture of idiopathic oedema which aetiologically does not seem to be always related to production of aldosterone

Hokfelt Prof Mach to return to the case which I have already discussed (p 133) in view of our findings of high 17 ketogenic steroids high blood pressure and low aldosterone while the patient was oedematous we think that in our case we are dealing with a different syndrome to that which you have observed As already mentioned we have some evidence in favour of pituitary tumour I wonder whether in view of the special mental disturbance which you have in your patient any of the old or new tranquillizing drugs have been tried?

Mach We did not try tranquillizing drugs since we got a very fair therapeutic response by simply increasing the fluid intake

Gross Prof Mach did you attempt an elimination of water in your patient in using prednisone and prednisolone?

Mach No we did not since a previous trial was not convincing

Gross You mentioned the fact that you were not able to depress the aldosterone secretion sufficiently in your patient to allow a normalization and a free salt intake Now what is really normal aldosterone secretion and can we reach conclusions concerning secretion on the basis of excretion studies? For instance Dr Garrod mentioned figures of the order of 23 µg as high normal values whereas Prof Mach considers 20–23 as definitely abnormal and Prof Wolff showed that 60 and 100 µg per day are eliminated in pregnancy without clinical symptoms of hyperaldosteronism or marked sodium retention Therefore I think high aldosterone excretion alone is not sufficient to explain all those pathological states and syndromes which have been discussed at this symposium and probably additional factors also play an important role

Mach I agree with Dr Gross that it is not one or two exceptionally high values of aldosterone which explain this pathological situation but rather the fact that after a salt load these patients are unable to decrease adequately their aldosterone output and thereby eliminate the supplementary salt intake This salt loading test giving information about a functional state has much more significance than a few isolated aldosterone values

J F Tait Whilst agreeing with the general remarks of Dr Gross and Prof Mach regarding for instance the importance of sodium loading in classifying normals I think Dr Gross is being a little pessimistic about the discrepancy between various centres of research in their normal means and ranges This would appear to be mainly a technical problem of the hydrolysis of the 3 oxo hormone conjugate and if this process is standardized and proper statistical criteria used there seems to be little reason why we should not get good agreement between various groups

Muller Concerning the cardiac patients our experience corresponds to that of Prof Wolff and Dr Bartter We find a group of patients who instead of responding to salt restriction with a sodium and water diuresis further increase their aldosterone excretion which was generally already high before sodium was restricted On the other hand there are cardiac patients who lose their oedema while on a restricted salt intake They are the ones whose urinary aldosterone is generally not abnormally high to begin with and during the whole period when they are eliminating their oedema it either stays low or increases very insignificantly Once the patient is without oedema the urinary aldosterone increases because of the restricted sodium intake We explained this difference in behaviour in an earlier paper (1956 *Schweiz. med Wschr* 86 1335) in the same way that Prof Wolff does in his present study i e whether the patient can (or cannot) mobilize his oedema fluid from the periphery into the vascular channels and thereby maintain the same intravascular volume and renal plasma flow despite a continuous external sodium and water loss through the kidney and sometimes by perspiration

The second point I would like to discuss is the influence of prednisone on oedema Since we have already reported our experience with prednisone in cardiac patients (1956 *Schweiz. med Wschr* 86 1362) I only summarize our findings and then discuss in more detail two patients with nephrotic syndrome In general we found a good correlation between the decrease in urinary aldosterone and the increased water and sodium diuresis in our cardiac patients on prednisone However the diuretic response is variable and unpredictable which indicates that prednisone lowers aldosterone not directly but indirectly via volume changes as well as by facilitating water diuresis and internal fluid shifts This decrease in aldosterone is peculiar to the oedematous patient since the normal subject does not noticeably change his urinary aldosterone excretion with prednisone

Recently we had the opportunity to study two children with nephrotic syndrome Fig 3 shows the pertinent data in one of these patients a 12 year old girl with longstanding disease Several points are of interest (1) Whereas prednisone immediately increases the water diuresis it influences the urinary sodium excretion only after 11 days (2) After a first 7 day sodium and water diuresis with a 7 kg weight loss the renal tubular reabsorption of sodium is again complete and the weight stabilized despite continuing prednisone therapy (3) On discontinuing prednisone a second diuresis begins with a concomitant weight loss of 3 kg. (4) There seems to be no correlation between plasma sodium and urinary sodium excretion (5) The sodium excretion is exactly the mirror image of the urinary aldosterone pattern However despite the immediate lowering of the elevated aldosterone values sodium appears in the urine only on the 11th day when the aldosterone has reached a level of 60 µg /24 hours Because of a second increase in aldosterone sodium reabsorption is again complete (6) The extremely elevated aldosterone values if we calculate on the basis of our usual recovery rate would correspond to a 20 to 30 mg daily secretion of aldosterone Since this is highly unlikely we would like to postulate an abnormality in aldosterone metabolism in this child The fact that this patient eliminates between 40 and 180 m-equiv of sodium a day despite urinary aldosterone levels as high as 60 and 17 µg /24 hours also indicates an abnormal metabolism with a different urinary recovery of aldosterone The second patient a 3 year old boy shows essentially the same clinical and metabolic evolution but the urinary aldosterone levels

are lower (196 µg./24 hours) We are unable to explain why both of these patients decreased their urinary aldosterone output during prednisone therapy as well as on discontinuing the drug However the urinary sodium

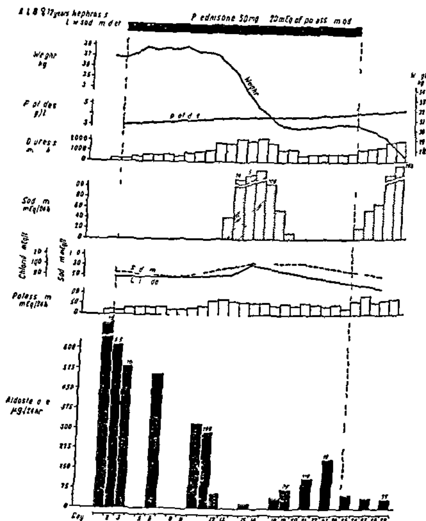


Fig 3 (Muller) Influence of prednisone on body weight water diuresis and urinary sodium, potassium and aldosterone in a 12 year-old girl with nephrosis. Note reciprocal behaviour of urinary aldosterone and sodium.

loss which occurred on both occasions seems to be in a direct and causal relationship to the lowered aldosterone

Giroud You are certainly aware of the experiment of Dr Singer (1957 *Endocrinology* 60, 420) who definitely proved that in an experimental nephrotic syndrome induced in rats by an aminonucleoside of puromycin,

the secretion of aldosterone in the adrenal vein blood is strikingly increased In an attempt to investigate further the mechanism of this overproduction of aldosterone Mr Das Gupta in our laboratories has been studying the aldosterone secretion of such nephrotic rats after treatment with cortisone and prednisolone It was observed that after 5 injections of cortisone or prednisolone in a daily dose of 500 and 250 μg /100 g of body weight respectively the aldosterone content of the adrenal vein blood decreased to normal control values

Vesin Since 1954 we have treated approximately 60 patients with oedematous cirrhosis and also with cardiac nutritional and renal oedemas with cortisone and more recently with prednisone We have investigated in many cases and repeatedly the blood volume by means of the Chicago blue and ^{51}Cr techniques We found in these cirrhotics and in other patients despite the frequent existence of very marked hypoproteinaemia a very great increase in the blood volume and that at a time when they were actively retaining sodium When we determined aldosterone in the urine we found high values (Our technique was similar to that of Neher and Wettstein) This coincidence of an increased blood volume with high levels of aldosterone and active sodium retention is surprising and apparently contradictory However we think that the formation of oedema whatever its aetiology is always accompanied by hypovolaemia which then secondarily increases the aldosterone secretion and sodium retention by a homeostatic mechanism with restoration of blood volume often over normal figures However there is a continuous shift of water and electrolytes from the blood into the interstitial space (expanding oedema) which induces a decrease in volume calling for continuous hypersecretion of aldosterone and antidiuretic hormone In other words we have a situation where a homeostatic mechanism increases the very trouble which it is supposed to correct namely oedema We gave the name disease of homeostasis to this situation (Cattan R and Vesin P (1956) *Sem Hop Paris* 13 712)

The second point which I would like to discuss is the action of prednisone on aldosterone secretion in oedematous cirrhosis On the basis of Luetscher s observations in nephrosis with ACTH it seemed likely that prednisone would also decrease aldosterone in cirrhosis which then would allow the well known sodium and water diuresis which we have observed We have been able to confirm this hypothesis (Vesin P *et al* (1956) *Sem Hop Paris* 62 3205) Drs Muller and Bartter have made similar observations Tentatively we would like to explain this action of prednisone in the following way since the first and principal action of prednisone is a water diuresis as well as a frequent increase in sweat the normal sequence of events must be hypovolaemia following water depletion but it is immediately corrected by an inflow of water from the interstitial tissue where it is present in large amounts in view of the oedema This inflow can be explained by the rise concomitant to water depletion in blood osmolarity and protein oncotic pressure This mobilization of water therefore prevents the hypovolaemia and secondarily the increased aldosterone output which would be an obligatory event It is this possibility to mobilize water and salt which distinguishes the oedematous patient with his extra store of interstitial water from the normal subject since the latter will always increase his aldosterone output after a water and salt loss in order to prevent dehydration Therefore as long as the oedematous patient mobilizes his salt and water from the interstitial tissue under prednisone

treatment he will decrease his oedema and maintain his blood volume as we have observed and there will be no increase in aldosterone On the contrary the increased secretion of aldosterone will decrease and even return to normal or nearly normal figures most likely by reduction or suppression of the stimulus (hypovolaemia above a given threshold?) exciting the secretion of aldosterone (Farrell s cerebral centre?) Whatever the intimate mechanism of this inhibition of aldosterone hypersecretion following the hydroelectrolyte variations of the milieu interieur due to the action of prednisone we must emphasize the capital therapeutic significance of this fact because if aldosterone hypersecretion (and sodium retention) persists oedema can be controlled clinically more or less but not biologically and easily recurs therefore the ability of prednisone and other cortisone like steroids to reduce with frequency aldosterone hypersecretion in severe oedemas of all origins makes these steroids a major agent in the treatment of oedema (Vesin P and Cattan R (1957) *Sem Hop Paris* 2 67)

Gabrilove Since the question of oedema or lack of oedema is so closely interrelated with aldosterone I would like to mention a few pertinent observations A number of years ago when Soffer and I and our associates studied Cushing s syndrome we found that if we gave cortexone to normal patients following salt loading they retained salt whereas surprisingly enough a large percentage of patients with Cushing s syndrome diuresed the salt with this procedure At that time we found that those who diuresed salt so to speak had a normal serum sodium whereas most of those who behaved as normal subjects did had a serum sodium elevated above 145 m equiv /l Gaudino and Levitt and then our associates Levitt and Bader measured the extracellular fluid following the administration of cortexone and cortisone in dogs and human subjects and made a very interesting observation that the extracellular space increases for a period of 8 to 10 days and then in spite of continued therapy the extracellular fluid space contracts and goes back to normal We then confirmed these observations and went on to treat some of our patients with Cushing s syndrome In one patient who had one of these so-called paradoxical responses that we saw in Cushing s syndrome we measured the extra cellular space and found as we had hoped that following the administration of cortisone or cortexone the extracellular space promptly decreased in a period of 3 days When we took one of the patients who failed to respond in the usual orthodox Cushing manner and treated him similarly the extracellular space behaved in a fashion identical to that seen in normal subjects although to a lesser degree Now when we gave aldosterone to a normal subject we induced an increase in the extracellular space Due to the limitation of the amount of aldosterone available we only gave it for about 5 days In this connexion Dr Conn s observation in his patient with primary hyperaldosteronism is also interesting since he obtained a diuresis of sodium with ACTH and cortisone

I may also mention an early observation of ours In 1949 a patient was admitted because of oedema and hypertension We found a definite increase in the intravascular volume of approximately 30–35 per cent This patient s hypertension and oedema could not be explained on a renal or cardiac basis. The urinary excretion of the neutral 17 ketosteroids and of the formaldehydogenic corticoids was at the upper limit of normal However large quantities of Δ 3 5 androstadene 17-one were found in the urine The glucose tolerance test revealed a diminished glucose toler

ance We suspected an adrenal tumour This was found to be the case on presacral air insufflation Following removal of the tumour the oedema disappeared for a period of three months Since the tumour was malignant the symptoms recurred and the patient died. Since this did not look to us like an orthodox case of Cushing s syndrome our interpretation was that it might be a mineralocorticoid producing tumour

Luft We should not forget that there is a stage where we have a large expansion of the extracellular fluid which goes on for years but without oedema this is acromegaly Prof Querido has done some aldosterone determinations in acromegalic patients and he tells me that the values were within the normal range

GENERAL DISCUSSION

Hoet I should like to present the case of a 54-year old married farmer who was admitted to us because of severe hypertension paralysis and mild mental confusion Past history revealed severe polydipsia (more than 5 litres a day) polyuria nycturia progressive weakness and increasing tendency to fatigue throughout the previous year The initial physical examination blood and urine analyses are summarized in Table I

Table I

INITIAL PHYSICAL EXAMINATION AND LABORATORY VALUES

No features of Cushing s disease no rales or oedema ocular fundi KW Stage IV deep tendon reflexes absent blood pressure 270/160 mm Hg temperature 98 8 F ECG left ventricular hypertrophy

Blood values	*Urine values*
Blood count RBC 4 500 000/mm	Albumin 3 g /l
Hb 90 /	
WBC 21 700/mm.	Sediment granular casts
Eos 70/mm	RBC 10–15/field
	WBC 0–2/field
Urea 1 g /mille	
Fasting blood sugar 1 g /mille	Specific gravity 1015 Isosthenuria
Serum protein 6 2 g /	
albumin 48 /	Urea Cl 48 /
Hematocrit 45	PSP 28 / (in 2 hours)
Serum Na 139 m equiv /mille	
K 2 5 m equiv /mille	17 hydroxysteroids (2 3 1957) 10 mg /24 hours
Ca 5 m equiv /mille	17 ketosteroids (2 3 1957) 2 mg /24 hours
P 1 9 m equiv /mille	aldosterone (2 3 1957) 7 4 µg./24 hours
Cl 86 m-equiv /mille	17 hydroxysteroids (12 3 1957) 5 mg /24 hours
CO_2-combining power	17 ketosteroids (12 3 1957) 8 mg /24 hours
38 m equiv /mille	aldosterone (12 3 1957) 16 5 µg /24 hours

Conclusion metabolic alkalosis with hypokalaemia dehydration and haemoconcentration presumptive diagnosis primary hyperaldosteronism

Chemical analysis of the blood revealed the clinical picture of hypokalaemic hypochloraemic alkalosis In view of the patient s past history physical examination and metabolic alkalosis with hypokalaemia without extrarenal potassium loss a presumptive diagnosis of primary hyperaldosteronism was made The patient needed 200 m-equiv of potassium per day to prevent alkalosis and hypokalaemia. During this period urinary aldosterone determination revealed 16 5 µg /24 hours * Previous to this

* This determination was carried out through the courtesy of Dr R. Neher who has also kindly undertaken analysis of the steroids of the tumour in his laboratory

period one urinary aldosterone determination revealed 7 4 μg /24 hours The data is presented particularly in view of the electrolyte changes occurring during amphenone therapy (Figs 1 and 2)

More sodium and less potassium was excreted during amphenone therapy while the Na/K ratio in the urine rose from 0 6 to 1 48 After

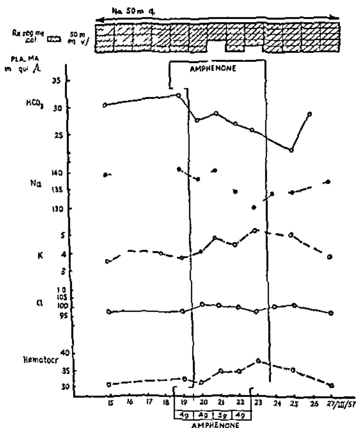


FIG 1 (Hoet) Changes in plasma sodium potassium, chloride and CO combining power as well as haematocrit on amphenone therapy in a 54 year-old man with Conn's syndrome

this period less sodium was excreted and the Na/K ratio dropped to 0 3 The blood chemistry changes were dramatic the CO_2-combining power dropped from alkalotic levels to low normal the serum sodium dropped also while the serum potassium went up from 3 7 to 5 3 m equiv /l When amphenone was discontinued the CO_2-combining power and the serum sodium rose while the serum potassium dropped On further investigation a presacral air insufflation revealed a normal kidney and

adrenal on the right side However on the left side there was a definite rounded mass visible in the suprarenal region We noticed the absence of a *normal kidney* The patient died as the result of a cerebrovascular accident shortly before the day scheduled for operation

The autopsy findings confirmed our clinical diagnosis There was a normal kidney and a normal adrenal weighing 7 5 g on the right side whereas on the left side the kidney was bilobar and atrophic and weighed 35 g There was a normal left adrenal (7 g) and an encapsulated adenoma

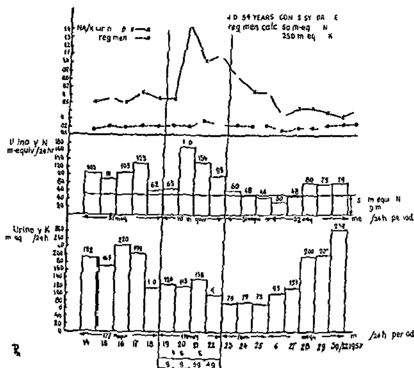


FIG 2 (Hoet) Changes in urinary sodium potassium and Na/K ratio on amphenone therapy in a 54-year old patient with Conn s syndrome

weighing 13 g was present on the same side The adenoma contained adrenal tissue resembling the tissue of the zona glomerulosa while the zona glomerulosa of both adrenals was not atrophic We might conclude that the hypokalaemic alkalosis was dependent upon adrenal corticosteroid secretion since amphenone administration inhibiting this secretion caused the pathological picture of blood electrolytes to revert to normal Amphenone also produced changes of the urinary excretion of sodium and potassium showing the biochemical effect of the inhibition of adrenal secretion The administration of amphenone made it possible to demonstrate the implication of the adrenal corticosteroid secretion in the disturbed

electrolyte balance It provides further evidence in support of the diagnosis of primary hyperaldosteronism or Conn s syndrome in this case where a small atrophic kidney was associated with an adrenal adenoma

Bartter I should like to present briefly some studies on two patients to illustrate two points

Fig 3 shows some data on a 13 year old boy who presented a history of hypertension of 18 months duration which had been discovered as an

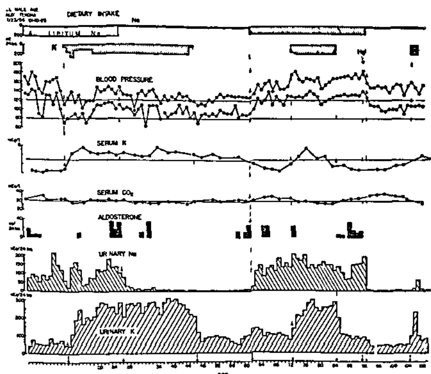


FIG 3 (Bartter) Sodium and potassium intake, blood pressure serum potassium and CO and urinary aldosterone sodium and potassium in a 13 year-old boy

incidental finding He had essentially no symptoms Eighteen months previously both adrenals had been explored for phaeochromocytoma it is partly for that reason that so extensive a study was carried out before re-exploration On admission he had hypertension a low serum potassium and a high serum CO_2 content Of the first four urinary aldosterone values only one was elevated (19 µg /24 hours) for the sodium intake From days 12–26 large loads of potassium were given to rule out potassium depletion as the cause of the low urinary aldosterone values Three out of five values were indeed above normal on this regimen From days 26–61

sodium was restricted to 9 m-equiv per day to rule out obligatory renal sodium loss as the cause of the high aldosterone output. Urinary sodium fell to very low values. Normal serum potassium and CO_2 values persisted without supplementary potassium as long as sodium was restricted.

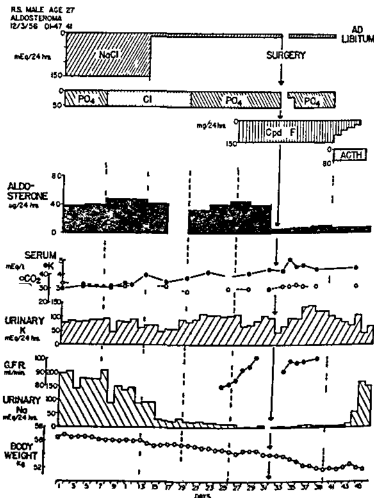


FIG 4 (Bartter) Sodium phosphorus and chloride intake serum potassium and CO and urinary aldosterone, sodium and potassium in a 27 year old man with aldosteroma Note rise of endogenous creatinine clearance (GFR) with cortisol

Aldosterone excretion was no higher after a month of sodium deprivation (days 57–61) than it had been on liberal sodium intake a finding which might support the notion that an autonomous tumour was producing aldosterone The remainder of the study shows return of hypokalaemic

alkalosis when sodium intake was increased its temporary improvement with potassium supplements and its persistence on low sodium intake until potassium was given Only two out of nine aldosterone values in this period were normal

Finally the patient was re-explored The adrenals were not enlarged one and four fifths were removed He is now completely normal on 1 g of sodium a day hypertensive on more approaching Addisonian crisis on less

Fig 4 shows some data on a 27 year-old man asymptomatic save for polyuria who presented because of hypertension discovered at a routine examination. Blood pressure was 240/160 CO_2 40 m-equiv /l and potassium 2 4 m-equiv /l when he was first seen 3 weeks before the study was begun

Urinary sodium fell to very low values with sodium restriction and serum potassium and CO_2 became normal. Compound F was given because Conn and others had suggested that it has a paradoxical effect on sodium in this syndrome There was no sodium diuresis The marked rise in endogenous creatinine clearance suggests that when sodium loss is seen in such patients it may result from an increase in filtered load Exploration revealed an adrenal adenoma He is now normal in every respect

Sodium restriction may be so effective in removing the symptoms of these patients that it should probably be instituted only after the other essential studies are done For over a year now I have been following with Dr John Eager Howard a patient who refuses further studies because she is completely asymptomatic if she avoids salt, despite marked hypertension aldosterone values in the hundreds and severe hypokalaemia alkalosis and tetany upon ingestion of sodium

Baulieu To return to the question of hypokalaemia of adrenal origin in Cushing s disease with adrenal non tumorous hyperplasia the relatively frequent finding of a marked hypokalaemia with low total exchangeable body potassium might suggest an additional hyperaldosteronism but in fact, the hypokalaemia of these Cushing s diseases is not obligatorily related to hyperaldosteronism (Table I p 222) as Garrod Simpson and Tait (1956 IV Int Congr Med) have also reported in two cases of tumours In Cushing s disease in general (as was shown also by the anatomical study in our cases) the zona glomerulosa is atrophic or normal and never hypertrophic like the fasciculata reticularis The low or normal aldosterone excretion might be related to this finding In the Cushing cases with hyperaldosteronism reported in the literature we noticed that oedema was always mentioned our own cases had no oedema

Stanbury When one meets cases such as that just described in which the features of Conn s syndrome are associated with a primary renal disease one is again compelled to ask whether an apparent primary aldosteronism is actually secondary hyperaldosteronism I should like to present the salient features of a case that will subsequently be published *in extenso* by Wrong and Gowenlock The patient was a man who developed at the age of 53 years the classical symptoms of Conn s

syndrome with thirst polyuria of 5 to 6 litres daily hypokalaemia and alkalosis He was hypertensive but the hypertension was only apparent if the blood pressure was measured in the legs The brachial arterial pressure was not significantly raised and the pulse pressure was narrow, we had to

Table I (Baulieu)

ALDOSTERONE EXCRETION IN CUSHING S DISEASE

Baulieu E E de Vigan M and Jayle M F (1957 Unpublished)

	Age	*Plasma potas sium*	*Blood p sure*	*Daily s d m intake* (1)	*T e t me t*	*Di sis ml /24 hours*	*17 keto-ste olds mg / 4 ho rs* (2)	*17 hydroxy st roids mg /24 h rs* (3)	*Cortisol µg /24 h rs* (4)	*Aldo-ste e µg /24 ho s* (4)
P f m. (5)	35	low	normal	free	0	850	84 0	69 0	>4 000	<3 (12)
L fem (6)	30	low	high	free	0	1050	6 2	24 6	300	0 2
				<10 m-equiv + >30 m-equiv potass um third day	0	800	—	—	330	3 2
				30 m-equiv (10)	0	400	—	—	220	1 8
C. fem. (7)	21	normal	high	free	0	920	19 0	21 0	>700	<3 (12)
				free	ACTH (9) second d y	870	(11)	(11)	—	<3 (12)
				free	ACTH (9) third day	1 00	(11)	(11)	—	<3 (12)
T male (8)	8	normal	high	free	0	470	12 9	34 5	350	<0 5
				<10 m-equ v third day	0	600	—	—	470	<0 5
				<10 m-equiv sixth d y	ACTH third day	850	(11)	(11)	460	2

(1) Approximately
(2) Ac d hydrolys s Zimmerman chromoge
(3) Enzymic hydrolys s P rte S lber method
(4) 24 ho r pH 1 hydr ly q nt tat ve paper chromatographic method Normal cortisol 0–150 µg and ldosteron 0 5–6 µg /24 hours
(5) Case rep rt Abely X D l ll M and Guiraud N (1957) *A Biol Cl n* 15 93
(6) S rvice J Deco rt, Hôp tal de la P t é P r s
(7) Th only case w thout a t mic l p oof of ad enal hype pla See dynamic st dies in de *G nnes L. Bricair H and Ba l u E E (1956) P M d* 64 1855
(8) Case port S l t J Br ca H Sée G J yle M F Baul eu, E E Ben Brah m R. and Ma J (1957) *Sem H p Ann Péd t l* 33 1599 p 233
(9) ACTH (Zn End pancrine Orga on) 40 I U per day intramuscularly
(10) Sod um cess not t l rated (oedema)
(11) Increase during oth r tests
(12) In these cases the technique showed only the upper lim t.

conclude that there was some vascular anomaly involving the upper limbs We knew from his army service records that this abnormality was present some 8 years previously and during his army service he also ex perienced some form of unilateral renal disease with renal pain and a palpable kidney When he came under our care several years later intravenous pyelography *showed a non functioning kidney on that side* Ascending pyelography and perirenal air insufflation demonstrated a sma

kidney and the latter investigation revealed apparently very small adrenal glands He had hyperaldosteronuria with an output of about 80 µg daily The excretion of aldosterone increased to 200 µg daily on treatment with potassium salts and it came down a little when prednisone was given in addition to potassium salts Surgical exploration confirmed our clinical findings He had unilateral renal disease with a very small kidney that had the histological appearance of Selye s endocrine kidney but which did not contain aldosterone and we wondered could Selye s endocrine kidney really be an endocrine kidney? The adrenal glands were rather small their total weight being about 5 g and nine tenths of the total adrenal weight was removed The urinary output of aldosterone fell at once and it has remained down (He is now excreting some 3–8 µg of aldosterone daily) All the symptoms have improved although the hypertension has not returned completely to normal Here is a patient who is known to have renal disease Is the renal disease responsible for a state of secondary hyperaldosteronism? And if it is responsible by what mechanism has the state of adrenocortical hyperfunction been brought about?

Garrod What was the histology of the adrenal?

Stanbury The histologists whom we consulted considered the adrenal to be normal in every particular

J F Tait Dr Stanbury I am still confused as to your distinction between primary and secondary primary aldosteronism

Stanbury I must apologise if my deliberately provocative use of the term secondary primary aldosteronism has led to confusion I invoked the concept in a pattern of clinical disease which is characterized not by potassium deficiency but by sustained sodium deficiency It is often clear from the history of patients with salt losing nephritis that they may have been severely deficient in sodium for a long period We are simply suggesting that after such a prolonged period of stimulation the adrenal cortex may continue to produce excessive quantities of aldosterone even when the causative stimulus has been removed

As to the unilateral renal disease there are on record several reports of patients with unilateral renal disease and hypertension in whom there has been excessive polyuria and thirst with polydipsia (e g Deming Q B (1954) *Arch int Med* 93 197 Margolin E G Merrill J P and Harrison J H (1957) *New Engl J Med* 256 58) Unfortunately neither the potassium level nor the CO_2 level in the plasma was measured in these patients. We have wondered whether the extreme polyuria experienced by some of these patients with unilateral renal disease and severe hypertension may not reflect a situation comparable to the one I have just summarized

Bartter Dr Schwartz and I have studied a syndrome which was found in two male patients who manifested essentially asymptomatic hyponatraemia They had the following points in common (1) progressive hyponatraemia with large urinary sodium losses which could be shown to develop without loss of body weight and with no clinical evidence of dehydration (2) urine persistently hypertonic to the serum (3) normal

adrenocortical function as judged from cortisol output response to ACTH and aldosterone output (4) normal renal function with normal to high glomerular filtration rates normal blood urea nitrogen values and prompt retention of sodium upon administration of sodium retaining steroids (5) mediastinal tumours (bronchogenic carcinoma) and finally (6) restoration of normal serum sodium values with decrease of urinary sodium excretion upon restriction of water

We suggest that the findings in these patients can best be explained by postulating that continuous inappropriate secretion of antidiuretic hormone resulted in some fashion from the disease state All the abnormal findings they showed as regards sodium and water can be reproduced in normal subjects by the continuous administration of pitressin and water as with these patients such subjects show no abnormalities of salt and water balance when water is withheld It appears that the urinary sodium loss which follows such expansion of body fluids results both from increase in filtration rate and from failure of aldosterone secretion to rise as it ordinarily does when with sodium depletion extracellular fluid volume contracts

Luft Prof Mach has asked me to take on the heavy task of summarizing this meeting I think that at the moment no task could be more difficult

When we came here there were many questions that we hoped would be answered We wanted to know where aldosterone is produced—in what part of the adrenal cortex We wanted to know simple things about the biogenesis of this compound We wanted to know if there is available data on the metabolism of aldosterone We wanted to know what controls aldosterone production and finally some of us clinicians had in mind all the clinical peculiarities that we meet in so-called primary and secondary hyperaldosteronism.

We have been furnished with beautiful new data on the chemical and biochemical side of the subject The presentations of Drs Neher and Giroud on the biogenesis and the presentations of Dr and Mrs Tait and co workers on the degradation of aldosterone add to the earlier very important contributions of these groups No doubt we shall soon get further data which will elucidate these important parts of the problem The paper of Drs Moolenaar and Querido gave rise to a very important and very healthy discussion on the methodology of the problem the determination of aldosterone in body fluids Many of us had the feeling during that discussion that we are rather far from any state of perfection and that we can expect new developments in this field This may remind us to be very careful when putting our aldosterone values in urine into clinical terms and trying to explain physiological and pathological conditions on the basis of these values only

The data presented by Drs Stahl Bartter and Muller on the physiological control of aldosterone production has stressed the significance of such factors as fluid shifts and of intravascular volume on the basic output of aldosterone and of the effect of posture on the control of the diurnal variations of aldosterone excretion. The physiological action of aldosterone has been elucidated by Drs Gross and Desaulles I should like

to emphasize the necessity of considering in the future other factors that might play a role in the control of aldosterone secretion Such factors have cropped up now and then in the discussion during these two days factors that may work on the cell alone and that we may without using their exact terms consider to be connected with shifts of water between the intracellular and extracellular compartments changes in the membrane potentials and so on Perhaps in the future we may consider such shifts and such changes in special areas of the body for instance in some centres of the hypothalamus It may be called wishful thinking to presume that we shall get any definite information along these lines in the next few years

The programme today has been devoted mainly to the clinical aspects of aldosterone We have been faced again with the usual problem of why primary aldosteronism differs from the secondary syndrome as regards oedema and potassium metabolism Dr Garrod clearly defined these problems For a clinician Dr Stanbury s diversion from aldosterone towards the kidney and its handling of potassium and the effect of potassium deficiency on renal function was a masterpiece of clinical and logical thinking based on exact studies Dr Morel s beautiful contribution has to be mentioned again

The last part of the day has again put us in a spot where we may only hope that in the near future this situation will be clarified Thanks to Profs Mach and Wolff we see clearly the great significance of future studies on the metabolism of water and electrolytes on the important clinical problem of oedema formation and how to handle the oedematous states

I began my review by summarizing some pertinent questions that were in our minds when we came here We have got some answers On the other hand at the present state of the study of aldosterone certain questions seem to be of critical importance at this time and this moment seems to be optimum for presenting them I would like to submit these questions to Prof Mach and to the group from Basel to form a basis for the next aldosterone meeting in Geneva and with a suggestion that we will have such a meeting once a year The first question would be which are the main excretory products of aldosterone since only such small amounts are excreted as aldosterone? The second question—again the old question why do the tumours produce syndromes different from those of cardiac failure and cirrhosis? And the third question is again an old one what is the mechanism of aldosterone control in oedematous states? Numerous hypotheses have been suggested for the explanation of this Two of them were very interesting ones but I think we agree that it remains for further experimentation to choose between them

We extend our deep gratitude to Prof Mach and Dr Muller and to Drs. Gross and Wettstein for having given us the opportunity to be members of this very interesting and important symposium

AUTHOR INDEX TO PAPERS

SUBJECT INDEX

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